An Anthrone-Based Kv7.2/7.3 Channel Blocker with Improved Properties for the Investigation of Psychiatric and Neurodegenerative Disorders

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ABSTRACT: A set of novel Kv7.2/7.3 (KCNQ2/3) channel blockers was synthesized to address several liabilities of the known compounds XE991 (metabolic instability and CYP inhibition) and the clinical compound DMP 543 (acid instability, insolubility, and lipophilicity). Using the anthrone scaffold of the prior channel blockers, alternative heteroarylmethyl substituents were installed via enolate alkylation reactions. Incorporation of a pyridazine and a fluorinated pyridine gave an analog (JDP-107) with an optimal combination of potency (IC_{50} = 0.16 μM in a Kv7.2 thallium flux assay), efficacy in a Kv7.2/7.3 patch clamp assay, and drug-like properties.
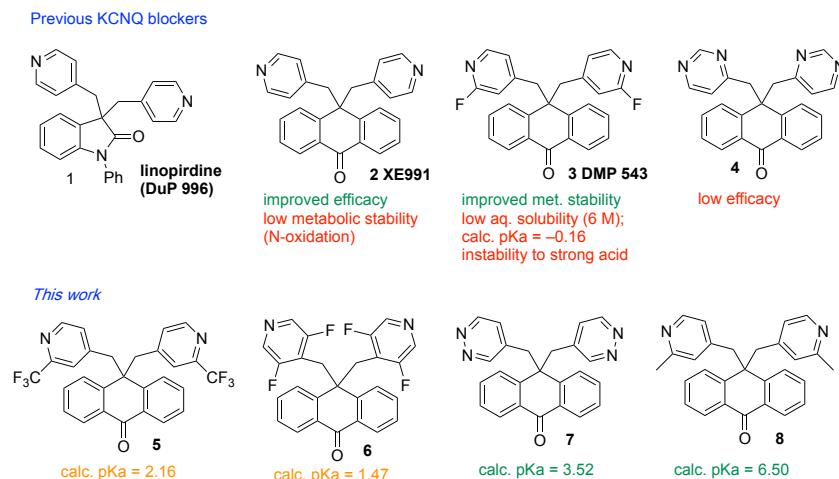
**Figure 1.** Previous and planned KCNQ channel blockers

1. Introduction

New and more efficacious treatments for schizophrenia are greatly needed, and this need has not yet been diminished by enormous investments in the clinical investigation of new drug targets.[1-3] One approach to schizophrenia drug discovery involves the repurposing of compounds that have at least demonstrated safety in clinical trials.[4] With this in mind, our interest in the chemical modification and redesign of bioactive natural and unnatural scaffolds led us to reexamine blockers of specific voltage-gated potassium channels of the family Kv7 (KCNQ). These are expressed in the nervous system, and two subtypes, KCNQ2 and KCNQ3, combine to form heteromeric channels that regulate the excitability of neurons via the so-called M-current.[5] Previously, certain compounds possessing a rigid carbo- or heterocyclic core with two pendant Lewis basic sites were demonstrated to enhance the release of acetylcholine (ACh) in neuronal tissue and were under clinical investigation as cognitive enhancers for the treatment of

dementia.[6-11] Linopirdine (**1**)[6,12-14] was well-tolerated but ineffective as a cognitive enhancer in a phase 3 trial with Alzheimer's patients.[15] Subsequent work led to the characterization of these compounds as blockers of Kv7 (KCNQ) potassium channels that control neuronal M-currents.[16-20] More recently, a patent by Ghasemzadeh reported that the KCNQ2/3 channel blockers XE991 (**2**)[7] and DMP 543 (**3**),[10,11] a compound also previously in clinical trials, could reverse the effects of PCP in a mouse model of schizophrenia, as measured by prepulse inhibition, social interaction time, and forced delayed alternation task.[21] The patent also claimed that such compounds could be used to attenuate the negative symptoms of schizophrenia or treat drug addiction. Very recently, Shi and Xie reported that XE991 acts as a neuroprotective agent in a mouse model of Parkinson's disease.[22] These findings prompted us to reexamine the use of KCNQ2/3 channel blockers for the potential treatment of neurological disorders, and to identify novel compounds with improved properties.

Beginning with patent applications first submitted in the 1980s,[23] researchers at DuPont Pharmaceuticals disclosed examples of scaffolds with α,α-bispyridylmethyl substituents as neuronal ACh releasers for potential use as cognitive enhancers (compounds **1** to **4**, Chart 1). Linopirdine and XE991 suffer from a lack of metabolic stability due to pyridine N-oxidation, which can be ameliorated by fluorination at the 2-position of the pyridine (DMP 543).[10] Two more recent examples of KCNQ2 blockers are UCL2077[24,25] and ML252,[26] at least one of which suffers from low metabolic stability.[26] Based on the lack of any significant safety/toxicity issues reported for linopirdine in the clinic,[15] and the potency and stability of DMP 543,[10] we focused our efforts on identifying novel anthrone-based KCNQ2/3 blockers that may have improved properties. In particular, DMP 543 has several liabilities, including low aqueous solubility (6 μM) and a lack of stability under acidic conditions.[27,28] Herein are described our efforts at addressing these issues with novel analogs (Chart 1, bottom).

The acidic instability of DMP 543 is likely due to a S_NAr mechanism whereby the pyridine is reversibly protonated prior to attack by water at the 2-position, followed by elimination of fluoride.[28] We reasoned that replacement of the 2-fluoro pyridine substituents with 2-trifluoromethyl (**5**) or 3,5-difluoro (**6**) could provide compounds that maintained KCNQ2/3 channel blocking activity and stability to pyridine N-oxidation while greatly improving stability under acidic conditions. We also reasoned that improved solubility may be feasible without compromising metabolic stability by using alternative heterocycles. A 4-pyrimidine analog of XE991 was reported by researchers at DuPont, but it was more than tenfold less potent (EC_{50} = 4.4 vs 0.4 μM), and possessed less than 1% of the efficacy of XE991 in an assay measuring ACh release.[7] Extensive SAR studies from researchers at DuPont have indicated that heteroatoms at the termini of both side chains are required for ACh release activity,[6,7,14] and we hypothesized that the pyridazine analog **7** could act as a hydrogen-bond acceptor and perhaps maintain potency and efficacy. We would expect it to have improved metabolic stability relative to XE991, improved solubility and acid stability relative to DMP 543,[29] and a decreased volume of distribution, as suggested by its calculated logP (clogP) that is 3 units lower than DMP 543 (2.9 versus 5.9, Figure 1). The 2-methylpyridine **8** was prepared as a control compound to compare in particular with the 2-trifluoromethyl analog **5**. Calculated pKa values of **5** to **8** suggest that they could all potentially undergo salt formation with HCl (not possible with DMP 543), which could lead to improved kinetic solubilities.

Scheme 1. Representative synthesis of bis-alkylated anthrone
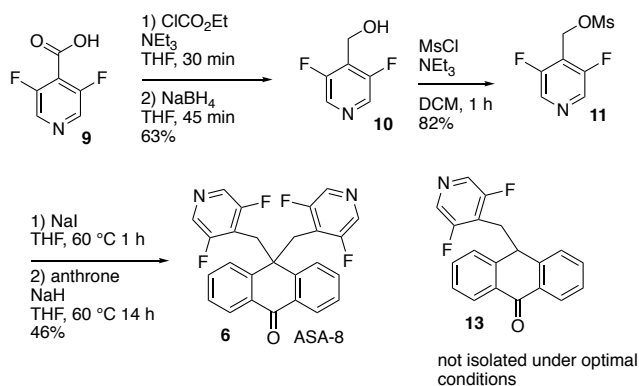

2. Results and discussion

Analogs **5–8** were synthesized by the alkylation of the anthrone scaffold with mesylate electrophiles, as reported by Pesti for the synthesis of DMP 543.[30] To provide suitable controls, we also synthesized DMP 543 itself. A representative synthesis is given in Scheme 1 for ASA-8 (**6**); synthetic details for the preparation of the other compounds is provided in the Experimental section. Pyridine carboxylic acid **9** was converted to a mixed anhydride, then reduced with LAH to yield alcohol **10**, which was converted to the benzylic mesylate **11**. The alkylation of anthrone with **11** to yield the final compound **6** was investigated by varying the base, additive, and solvent (Table 1). Initial optimization attempts explored different counterions for the *tert*-butoxide base with lithium and potassium *tert*-butoxide (entries 1–3) both giving similarly low yields even upon further heating. These reactions also showed the formation of monalkylated anthrone **13** by LC-MS as a prominent byproduct. Addition of 18-crown-6 ether (entry 4) to KO*t*-Bu did not improve the yield. Increasing amounts of NaI (entries 5 and 6) gave only a slight improvement in yield over entry 1. The use of NaH as the base (entry 7) was shown to significantly improve the conversion and yield of the second alkylation, as measured by remaining **13** after completion of the reaction.

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Table 1. Optimization of Anthrone Alkylation[a]
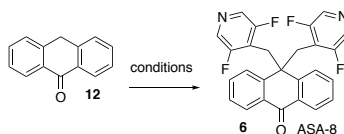
entry	base	additive[e]	solvent/temp.	yield[b,c]
1	LiOt-Bu	–	THF/60 °C	3%[b]
2	KOt-Bu	–	THF/60 °C	7%[b]
3	LiOt-Bu	–	THF/90 °C[d]	12%[c]
4	KOt-Bu	18-crown-6 (2.4 eq)	THF/60 °C	3%[b]
5	LiOt-Bu	NaI (1.5 eq.)	THF/60 °C	7%[c]
6	LiOt-Bu	NaI (2.0 eq.)	THF/60 °C	21%[c]
7	NaH	–	THF/60 °C	46%[b,c]

[a]Anthrone (0.055 mmol) and base (0.132 mmol) were mixed in THF (4.0 mL). The anthrone/base solution was added to a solution of the mesylate (0.110 mmol) and NaI (varying equivalents) in THF (4.0 mL), which had been heated at 50 °C for 3 h prior to addition. The reactions were then stirred at 60 °C for 12–16 h. See Experimental section for detailed protocol. [b]Isolated yield. [c]Yield measured by ^{1}H NMR using pentachloroethane as internal standard. [d]Reaction run in sealed tube in an oil bath heated to 90 °C.

Compounds **5–8** were tested for their ability to block currents at a concentration of 1.5 μM in tsA201 cells expressing Kv7.2/7.3, using a manual patch clamp protocol. Cells were held at –60 mV and stepped to –20 mV for 500 ms before returning to –60 mV. Time series were generated by repeating this protocol every 4 s. Kv7.2/7.3 currents were quantified from 'tail currents' upon stepping back to –60 mV. Tail currents were normalized to the initial values, and maximal channel block was determined by adding XE991 (20 μM) to the patch (taupe shaded boxes in Figure 2). The inhibition values for these preliminary screens are summarized in Table 2, along with XE 991 and DMP 543. Compounds **6** and **8** were the only novel compounds of the initial group that showed significant inhibition of Kv7.2/7.3 channel currents. **6** (65% inhibition) showed superior efficacy to DMP 543 (31% inhibition), and was comparable to the best-in-class compound XE991 (81%), at a concentration of 1.5 μM. Representative current traces are given for XE991, DMP 543 and **6** in Figure 2.
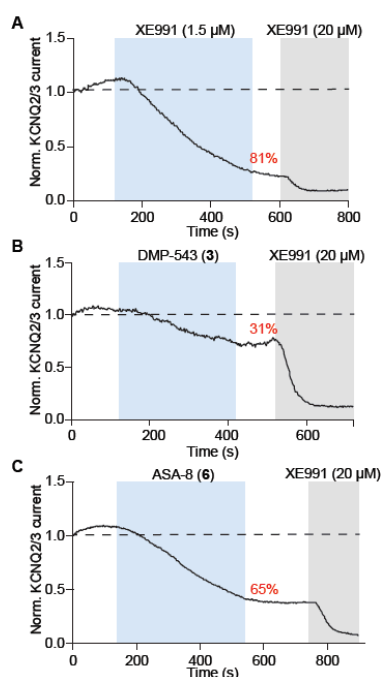
Figure 2. Representative electrophysiology traces of XE991 (**A**), DMP 543 (**B**), and **6** (**C**), in tsA201 cells expressing KCNQ2/3. XE991 (20 μM) was added late in each run to determine maximal channel inhibition.

Prior to in vitro metabolic stability studies, we measured the ability of our compounds to inhibit cytochromes P450 (CYPs), specifically the subtype 3A4 most associated with drug metabolism (Table 2). Using high concentrations (50 μM), all compounds inhibited CYP 3A4 to a significant degree, which is not surprising given the presence of pendant heterocycles in each compound capable of coordinating to heme iron centers. The bispyridazine **7** showed the lowest level of inhibition (28%), which is not unexpected given that it is a less basic heterocycle and a weaker metal ligand.

**Scheme 2.** Synthesis of pyridine-pyridazine JDP-107 (**18**)
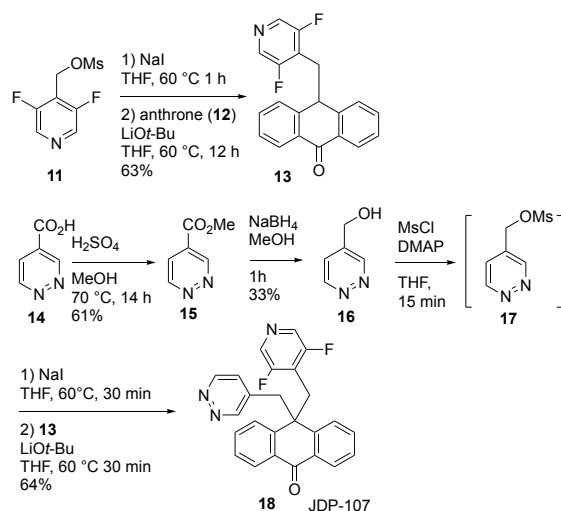
Table 2. K⁺ channel and CYP 3A4 inhibition data[a]

compound	Kv7.2/7.3 patch clamp inhib.[b] (@1.5 μM)	Kv7.2 flux IC_{50} (μM)[c]	hERG flux IC_{50} (μM)[d]	CYP 3A4 inhib.[e] (@50 μM)
XE991 (**2**)	81%	0.055	>32	NT
DMP 543 (**3**)	31%	0.048	32	82%
5	6%	5.6	inactive	95%
ASA-8 (**6**)	65%	0.20	12	97%
7	12%	10	inactive	28%
8	33%	0.54	inactive	92%
JDP-107 (**18**)	73%	0.16	>32	N.T.

[a]See Experimental section for assay protocols. N.T. = not tested. [b]Patch clamp assay using tsA201 cells expressing Kv7.2/7.3. [c]With HEK-293 cells stably expressing Kv7.2 channels. [d]With HEK-293 cells stably expressing hERG channels. [e]CYP 3A4 activities were determined using the Vivid CYP450 screening kit (Life Technologies, Carlsberg, CA, USA) according to the manufacturer's instructions.

To attempt to avoid the liability of CYP inhibition that could introduce drug-drug interactions upon clinical use, we considered the preparation of unsymmetrically alkylated anthrones. The pharmacophore for these channel blockers (ACh releasers) is well established to possess two pendant hydrogen bond acceptors, but we wished to determine if Kv7.2/7.3 could be effectively blocked by compounds possessing one stronger and one weaker hydrogen bond acceptor. Wilkerson reported that compounds in the oxindole series (e.g., linopirdine) can be highly efficacious acetylcholine release enhancers with one of the 4-pyridinylmethyl substituents replaced with an aliphatic ester or nitrile.[6] We reasoned that a compound containing one pyridazine could maintain efficacy and resistance to metabolic *N*-oxide formation, while potentially attenuating CYP inhibition. Such a compound is also expected to possess improved solubility and decreased volume of distribution relative to DMP 543, which is likely a desirable clinical profile if brain penetrance can be maintained.[10]

The synthesis of the pyridine-pyridazine **18** (JDP-107) is outlined in Scheme 2. Pyridazine-4-carboxylic acid (**14**) was converted to the methyl ester **15**, then reduced with NaBH₄ to generate alcohol **16**. Anthrone (**12**) was sequentially alkylated with mesylate **11**, then the mesylate **17** generated in situ from **16**, thus generating the desired analog **18**.
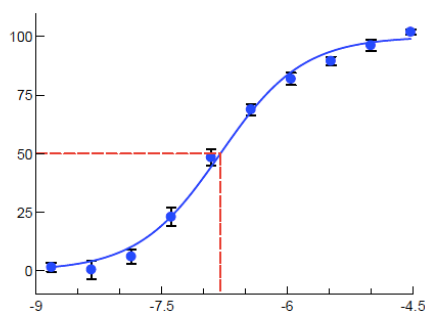
Figure 3. Concentration-response for JDP-107 (**18**) in Kv7.2 thallium flux assay. X-axis = log(concentration JDP-107 (M)); Y-axis = percent inhibition. Error bars indicate standard error of the mean (SEM).

Next, the compounds were tested in a thallium flux assay using HEK-293 cells stably expressing Kv7.2 or hERG channels (Table 2). [31] Compounds with sub-micromolar potencies showed similar maximal inhibition of Kv7.2, including XE991. DMP 543 was the most potent compound tested, with IC_{50} = 0.048 μM, very similar to XE991 (0.055 μM). The bis-trifluoromethylpyridine analog **5** was ~100-fold less potent in the Kv7.2 assay, and showed minimal inhibition in the patch clamp KCNQ2/3 assay. The bis-pyridazine **7** also showed only moderate inhibition of Kv7.2 (IC_{50} = 10 μM). The bis-methylpyridine analog **8** showed better potency in the Kv7.2 assay (IC_{50} = 0.048 μM). Pleasingly, the bis-(3,5-difluoropyridine) **6** showed good inhibition of Kv7.2 (IC_{50} = 0.20 μM) to go with its promising electrophysiology results. Comparable inhibition was also measured with the mixed pyridine/pyridazine analog JDP-107 (**18**) (Figure 3, IC_{50} = 0.18 μM), within approximately 3-fold the potency of DMP 543. This confirmed our hypothesis that effective inhibition may be obtained with one more basic and one less basic moiety which presumably act as H-bond acceptors within the channel. **18** also showed only minimal inhibition of the hERG channel, with no inhibition observed at 1 μM and an IC_{50} > 30 μM. **6** showed a significantly higher level of hERG inhibition, with IC_{50} = 12 μM. Importantly, it was also the most efficacious of the new analogs in the Kv7.2/7.3 patch clamp assay, with a maximal inhibition of 73%, significantly better than DMP 543 (31%).

The success of the inhibitors **6** and **18** prompted us profile their drug-like properties in comparison to DMP 543 (Table 3). Both DMP 543 and **18** inhibit CYP 3A4 substantially at a concentration of 10 μM, which could make for possible drug-drug interactions in human subjects. Both compounds also show decent liver microsomal stability, with DMP 543 showing the better stability after a 1 h incubation (97% vs 84% remaining). To compare the acid stability of compounds, DMP 543, **6,** and **18** were subjected to strongly acidic conditions (CH_3CN/0.2N aq. HCl). Compounds **6** and **18** showed no degradation, and DMP 543 showed some degradation (83% remaining after 24 h), based on HPLC analysis.

Table 3. Comparison of XE991, DMP 543, ASA-8 (6), and JDP-107 (18)[a]

	XE991	DMP 543	ASA-8 (6)	JDP-107 (18)
Kv7.2/7.3 patch clamp inhib. (@ 1.5 μM)[b]	81%	31%	65%	73%
Kv7.2 flux IC_{50}[c]	0.055 μM	0.048 μM	0.20 μM	0.16 μM
hERG flux IC_{50}[d]	>32 μM	32 μM	12 μM	>32 μM
CYP 3A4 inhib. (@ 10 μM)[e]	N.T.	79%	N.T.	90%
metabolic stability (human liver microsomes)[f]	N.T.	97%	N.T.	84%
acid stability[g]	N.T.	83%	>99%	>99%
ClogP[h]	4.86	5.93	5.43	4.17
calc. pKa[h]	5.77	–0.16	1.47	2.91
tPSA[i]	41.79	41.79	41.79	54.15
CNS MPO score[j]	4.0	3.6	3.4	4.0
kinetic solubility[k]	N.T.	22 μM	30 μM	27 μM
cytotoxicity (CC_{50}, hepG2 and HEK-293 cells)[l]	N.T.	132 μM; 77 μM	114 μM; 79 μM	>150 μM
Off-target	N.T.	6 (α_{1A} K_i	2 (PBR	5 (D3 K_i

| activities[m] | | = 1.1–3.1 μM; σ_1 K_i = 3.9 μM) | K_i = 2.5 μM; σ_1 K_i = 1.8 μM) | > 10 μM; σ_1 K_i > 10 μM) |

[a]See Experimental section for assay protocols. N.T. = not tested. [b]Patch clamp assay using tsA201 cells expressing Kv7.2/7.3. [c]With HEK-293 cells stably expressing Kv7.2 channels. [d]With HEK-293 cells stably expressing hERG channels. [e]CYP 3A4 activities were determined using the Vivid CYP450 screening kit (Life Technologies, Carlsberg, CA, USA) according to the manufacturer's instructions. [f]With human liver microsomes; percent remaining after 1 h. [g]Percent remaining based on relative HPLC peak areas (254 nm) after heating at 60 °C in 1:1 MeCN:0.2N aq. HCl for 24 h at a concentration of 0.3 mM. [h]Calculated with ChemAxon MarvinSketch v.18.3. [i]Calculated with ChemDraw Prime v.16.0. [j]Central Nervous System Multiparameter Optimization desirability score, calculated according to the spreadsheet published by Wager et al.[32] [k]Using 2.5% DMSO/water. [l]Measured using CellTiter-Glo® assay. [m]Defined as the number of targets (of 40, mostly GPCRs) which show >30% binding at a concentration of 10 μM. These studies were performed by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP).

To estimate the potential for our compounds to be safe CNS-active compounds, we used Pfizer's CNS multiparameter optimization desirability tool (CNS MPO), a freely available spreadsheet.[32] For each molecule, ClogP, ClogD, total polar surface area (tPSA), molecular weight, number of hydrogen-bond donors, and calculated pKa were determined and input into the spreadsheet to determine the CNS MPO scores. ClogP, ClogD, and pKa were calculated with ChemAxon MarvinSketch v.18.3, and tPSA was calculated with ChemDraw Prime v.16.0. The clinical compound DMP 543 possesses a score of 3.6 out of a possible 6, with 6 being the score for compounds with the most desirable properties for CNS drug development. The attenuated lipophilicity of **18** gave it an improved score of 4.0. However, there was not a substantial increase in kinetic aqueous solubility with the addition of a pyridazine to the scaffold (22 μM for DMP 543 vs 28 μM for **18**). Both DMP 543 and **18** showed low cytotoxicity with hepG2 cells using the CellTiterGlo® assay, with **18** showing no measurable cytotoxicity.

Finally, DMP 543, **6**, and **18** were submitted to the Psychoactive Drug Screening Program for off-target screening against a panel of mostly GPCRs, using competitive radioligand binding assays.[33] JDP-107 showed >30% inhibition of 5 of 40 targets (mostly GPCRs); K_is were measured to be >10 μM for the D3 and σ1 receptors. We conclude that at this stage, it has a clean receptor off-target binding profile for in vivo CNS studies.

3. Conclusions

We have determined that the anthrone scaffold of Kv7 inhibitors can tolerate replacements of pendant pyridine moieties with alternative heterocycles, and that such compounds could have an improved range of properties more suitable for in vivo and clinical studies. Specifically, we identified the pyridazine-containing analog **18** (JDP-107) that possesses comparable potency to DMP 543 in a Kv7.2 assay, superior efficacy in the Kv7.2/7.3 patch clamp assay, superior acid stability, and attenuated lipophilicity, though solubility and CYP inhibition continue to be liabilities of this compound class.

## 4. Experimental section

4.1 Synthetic chemistry

All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on Varian 300 MHz or 400 MHz spectrometers as indicated. Proton and carbon chemical shifts are reported in parts per million (ppm; δ) relative to tetramethylsilane, CDCl$_3$ solvent, or CD$_3$OD (^{1}H δ 0, ^{13}C δ 77.16, or ^{13}C δ 49.00, respectively). NMR data are reported as follows: chemical shifts, multiplicity (obs = obscured, app = apparent, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, sxt = sextet, m = multiplet, comp = complex overlapping signals); coupling constant(s) in Hz; integration. Unless otherwise indicated, NMR data were collected at 25 °C. Flash chromatography was performed using Biotage SNAP cartridges filled with 40–60 μm silica gel, or C18 reverse phase columns (Biotage® SNAP Ultra C18 or Isco Redisep® Gold C18Aq) on Biotage Isolera systems, with photodiode array UV detectors. Analytical thin layer chromatography (TLC) was performed on Agela Technologies 0.25 mm glass plates with 0.25 mm silica gel. Visualization was accomplished with UV light (254 nm) and aqueous potassium permanganate (KMnO$_4$) stain followed by heating, unless otherwise noted. Tandem liquid chromatography/mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-2020 with autosampler, photodiode array detector, and single-quadrupole MS with ESI and APCI dual ionization, using a Peak Scientific nitrogen generator. Unless otherwise noted, a standard LC-MS method was used to analyze reactions and reaction products: Phenomenex Gemini C18 column (100 x 4.6 mm, 3 μm particle size, 110 A pore size); column temperature 40 °C; 5 μL of sample in MeOH or CH$_3$CN at a nominal concentration of 1 mg/mL was injected, and peaks were eluted with a gradient of 25−95% CH$_3$CN/H$_2$O (both with 0.1% formic acid) over 5 min., then 95% CH$_3$CN/H$_2$O for 2 min. Purity was measured by UV absorbance at 210 or 254 nm. High-resolution mass spectra were obtained at the University of Wisconsin-Milwaukee Mass Spectrometry Laboratory with a Shimadzu LCMS-IT-TOF with ESI and APCI ionization or from the University of Cincinnati Environmental Analysis Service Center with an Agilent 6540 LCMS with accurate mass Q-TOF. IR spectra were obtained as a thin film on NaCl or KBr plates using a Thermo Scientific Nicolet iS5 spectrometer.

(3,5-difluoropyridin-4-yl)methanol (10). 3,5-Difluoropyridine-4-carboxylic acid (0.510 g, 3.14 mmol) was added to an oven dried 250 mL round bottom flask. The flask was sealed with a septum, purged with nitrogen, and anhydrous THF (50 mL) was added. Next, triethylamine (0.542 mL, 3.77 mmol) was added via syringe. The solution was sonicated until it became homogeneous, then ethyl chloroformate (0.372 mL, 3.77 mmol) was added. The reaction was stirred for 30 min. before being filtered through a short pad of Celite® into a separate oven dried 250 mL round bottom flask. $NaBH_4$ (0.261 g, 6.91 mmol) was added followed by MeOH (5 mL). The reaction was stirred for 45 min. at room temperature. A sample aliquot was taken from the reaction, dissolved in HPLC grade MeCN (1 mL), and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with sat. ammonium chloride and diluted with EtOAc (150 mL). The organic layer was washed with DI water (50 mL) and brine, then dried over sodium sulfate and condensed to give a yellow oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–12% MeOH/DCM gradient) to give the title compound as a clear semisolid (0.285 g, 63%). This compound has been previously reported and characterized (CAS# 924649-16-1). ^{1}H NMR (300 MHz, CDCl$_3$) δ = 4.81 (s, 2H), 8.28 (s, 2H) 8.30 (s, 2 H).

(3,5-difluoropyridin-4-yl)methyl methanesulfonate (11). Alcohol **10** (0.420 g, 2.89 mmol) was added to an oven dried 250 mL round bottom flask, followed by DCM (80 mL). Mesyl chloride (0.365 g, 3.18 mmol) was then added followed by triethylamine (0.444 mL, 3.18 mmol). The reaction stirred for 4 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL) and washed with water (3 x 30 mL). The aqueous layer was saturated with NaCl and extracted with EtOAc (3 x 40 mL) to give a light brown oil (0.475 g, 82%). The crude material was advanced without further purification or analysis.

10,10-bis[(3,5-difluoropyridin-4-yl)methyl]-9,10-dihydroanthracen-9-one (6). The mesylate **11** (0.010 g, 0.045 mmol) was added to an oven dried 8 mL reaction vial followed by NaI (0.003 g, 0.023 mmol). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. The solution was placed in an oil bath and heated to 60 °C for 1 h. Anthrone (0.004 g, 0.022 mmol) was added to a separate oven dried 4 mL reaction vial, followed by NaH (0.002 g, 0.054 mmol). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. After 1 h, the resulting anthrone anion solution was added to the mesylate/iodide solution under nitrogen dropwise over 2 min at 60 °C, and the reaction was stirred for 14 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge, 0–12% MeOH/DCM gradient) to give the title compound as a light brown oil (0.005 g, 46 %). ^{1}H NMR (400 MHz, CDCl$_3$) δ = 3.90 (s, 4H) 7.45 (t, *J* = 7.6 Hz, 2H) 7.66 (t, *J* = 7.6 Hz, 2H) 7.86 (d, *J* = 7.8 Hz, 2H) 7.93 (s, 4H) 8.19 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, CD$_3$OD) δ = 30.8, 38.7, 123.3 (t, *J*= 16.7 Hz), 127.5, 128.9, 130.1 (t, *J*=1.8 Hz), 132.5, 134.5,134.6 (dd, *J*=27.2, 3.8), 145.3, 184.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ = −126.8; HRMS (ESI$^+$) calcd for $C_{26}H_{17}F_4N_2O$ [M+H]$^+$ 449.1272, found 449.1257.

**Scheme 3.** Synthesis of 2-trifluoromethyl pyridine analog **5**
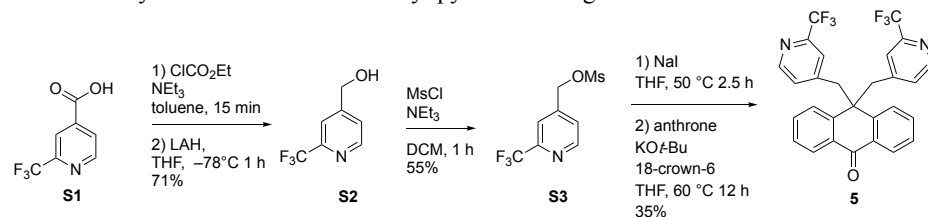
[2-(trifluoromethyl)pyridin-4-yl]methanol (S2). 2-(Trifluoromethyl)pyridine-4-carboxylic acid (0.206 g, 1.04 mmol) was added to an oven dried, nitrogen purged 4 mL vial containing anhydrous toluene (6.0 mL). Triethylamine (0.152 mL, 1.09 mmol) was added as a solution in anhydrous toluene (6.0 mL). Next, ethyl chloroformate (0.104 mL, 1.088 mmol) was added via syringe and the reaction stirred for 15 min. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the formation of the mixed anhydride. The solution was filtered through syringe filter (0.22 μm PVDF filter) and condensed to an oil. The oil was dissolved in anhydrous THF (6.0 mL) under nitrogen. This solution was added dropwise to a 20 mL reaction vial containing LAH (0.043 g, 1.09 mmol) in anhydrous THF (4.0 mL) cooled to –78 °C using a dry ice in acetone bath, then stirred for 1 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was warmed to 0 °C and quenched with sat. sodium sulfate solution (2 mL). The solution was filtered through a pad of Celite® and condensed to give a yellow oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO_2 cartridge; 0–100% EtOAc/hexanes gradient) to yield the title compound as a colorless oil (0.132 g, 71%). This compound has been previously reported and characterized (CAS# 131747-61-0) ^{1}H NMR (400 MHz, CDCl$_3$) δ = 2.66 (br. s, 1H), 4.85 (s, 2H), 7.50 (d, *J*=5.1 Hz, 1H), 7.72 (s, 1 H), 8.67 (d, *J* = 5.1 Hz, 1H).

[2-(trifluoromethyl)pyridin-4-yl]methyl methanesulfonate (S3). Alcohol **S2** (0.132 g, 0.745 mmol) was added to an oven dried 20 mL reaction vial, followed by DCM (10 mL). MsCl (0.063 mL, 0.820 mmol) was added via syringe followed by triethylamine (0.114 mL. 0.820 mmol), and the reaction stirred for 1 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL) and washed with water (3 x 40 mL). The aqueous layer was saturated with NaCl and re-extracted with EtOAc (3 x 20 mL).

The combined organic layers were washed with brine before being condensed to give the product as a yellow oil (0.130 g, 55%). The crude product was pushed forward without further purification or analysis.

10,10-bis({[2-(trifluoromethyl)pyridin-4-yl]methyl})-9,10-dihydroanthracen-9-one (5). Mesylate **S2** (0.132 g, 0.464 mmol) was placed in an oven dried 20 mL vial followed by sodium iodide (0.034 g, 0.222 mmol). The vial was sealed and purged with nitrogen before anhydrous THF (8.0 mL) was added. The solution was heated to 50 °C for 2.5 h. 18-crown-6 (0.367 g, 1.36 mmol) was dissolved in THF (3.0 mL) in a separate nitrogen purged 4 mL vial containing 4 Å molecular sieves. The solution was stirred for 1 h before being added by syringe to a 20 mL vial containing anthrone (0.040 g, 0.202 mmol) in THF (2.5 mL). Next, potassium *tert*-butoxide (0.070 g, 0.605 mmol) was added as a solution in THF (2.5 mL). The anthrone anion solution was added to the mesylate/iodide solution dropwise over 10 min. at 50 °C, and the reaction was stirred for 12 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL) and washed with brine (5 x 20 mL). The organic layer was condensed to give an orange oil, then dissolved with DCM and purified by flash chromatography (5 g SiO$_2$ cartridge; 0–60% EtOAc/hexanes gradient) to give the title compound as a clear oil (0.036 g, 35%). ^{1}H NMR (300 MHz, CDCl$_3$) δ = 3.84 (s, 4H), 6.35 (d, *J* = 5.0 Hz, 2H), 6.59 (s, 2H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.87 (t, *J* = 7.6 Hz, 2H), 8.04 (d, *J* = 8.2 Hz, 2H) 8.09 - 8.18 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ = 48.2, 49.4, 119.45, 121.5, 121.6, 121.6, 121.6, 123.1, 126.8, 127.1, 128.4, 128.6, 132.7, 134.0, 142.8, 146.7, 147.4, 147.8, 149.3, 181.5; ^{19}F NMR (376 MHz, CDCl$_3$) δ = –69.1; ; IR (film) 1661, 1602, 1326, 1178, 1135, 1117, 1088, 707, 687 cm^{-1}; HRMS (ESI$^+$) calcd for C$_{28}$H$_{18}$F$_6$N$_2$O [M+H]$^+$ 513.1396, found 513.1383.

Scheme 4. Synthesis of pyridazine analog **7**
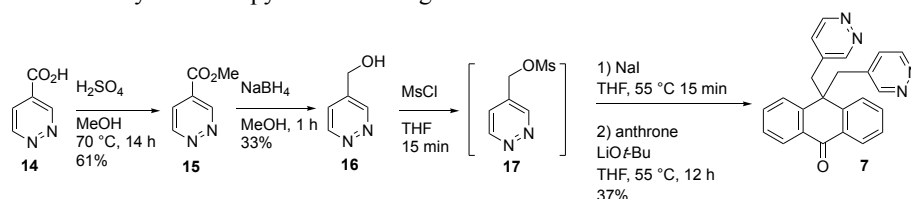
methyl pyridazine-4-carboxylate (15). Pyridazine-4-carboxylic acid 0.470 g, 3.79 mmol) was added to an oven dried 15 mL pressure flask, followed by anhydrous methanol (10 mL) and concentrated sulfuric acid (0.244 mL, 4.36 mmol). The flask was sealed and heated at 70 °C for 14 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (150 mL), then washed with half sat. aq. sodium bicarbonate solution (15 mL). The aqueous layer was then re-extracted with EtOAc (50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and condensed to give the desired product as an off-white semi-solid (0.262 g, 52%). The crude product was advanced without further purification. This compound has been previously reported and characterized (CAS# 34231-77-1). ^{1}H NMR (300 MHz, CDCl$_3$) δ = 3.98 (s, 3H), 7.95 (dd, *J* = 5.1, 2.2 Hz, 1H), 9.40 (d, *J* = 5.0 Hz, 1H), 9.63 (s, 1H).

(pyridazine-4-yl)methanol (16). The methyl ester **15** (0.133 g, 0.963 mmol) was added to an oven dried 20 mL reaction vial followed by methanol (1.2 mL). Sodium borohydride (0.073 g, 1.93 mmol) was added and the reaction stirred for 1 h. A sample aliquot was taken from the reaction, quenched with sat. ammonium chloride, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with sat. ammonium chloride and condensed to dryness to give a white solid. After condensing, DCM (100 mL) was added to the flask and the white solids were broken up and sonicated for 10 min, then vacuum filtered through a paper filter. The mother liquor was concentrated to give a yellow oil. The oil was dissolved with DCM and purified by flash chromatography (5 g SiO$_2$ cartridge; 0–12% MeOH/DCM gradient) to give the title compound as a yellow oil (0.035 g, 33%). This compound has been previously reported and characterized (CAS# 50901-43-4). ^{1}H NMR (300 MHz, CDCl$_3$) δ = 4.85 (s, 2H), 7.60 (dd, *J* = 5.3, 2.4 Hz, 1H), 9.06 (d, *J* = 5.3 Hz, 1H), 9.15 (s, 1H).

10,10-bis[(pyridazin-4-yl)methyl]-9,10-dihydroanthracen-9-one (7). Alcohol **16** (0.030 g, 0.272 mmol) was added to an oven dried 8 mL vial followed by DMAP (0.017 g, 0.136 mmol). The vial was capped and purged with nitrogen before anhydrous THF (7.0 mL) was added. Next, MsCl (0.021 mL, 0.272 mmol), then NEt$_3$ (0.038 mL, 0.272 mmol) were added via syringe. The reaction was stirred at room temperature for 15 min. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The mesylate solution was syringed out of the reaction vial under nitrogen and filtered through a syringe filter (0.22 μm PTFE filter) into a separate sealed, nitrogen purged 20 mL vial containing NaI (0.031 g, 0.201 mmol). The vial was sonicated for 2 min., then heated in an oil bath at 55 °C for 15 min. In a separate oven dried 8 mL reaction vial, anthrone (0.023 g, 0.118 mmol) and LiO*t*-Bu (0.024 g, 0.0242 mmol) were added. The vial was sealed and purged with nitrogen, then THF (7.0 mL) was added via syringe. The solution was sonicated until it became homogeneous. The resulting anthrone anion solution was added to the mesylate/iodide solution over 5 min., then stirred for 12 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was cooled to room temperature and quenched with brine, then diluted with EtOAc (100 mL). The organic layer was separated, then washed with brine (3 x 20 mL) ,dried over sodium sulfate, and condensed to give a brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO$_2$ cartridge; 0–12% MeOH/DCM gradient) to give the title compound as a brown oil (0.017 g, 37%). ^{1}H NMR (300 MHz, CD$_3$OD) δ = 4.01 (s, 4H), 6.55 (br s, 2H), 7.57 (t, *J* = 7.5 Hz,

2H), 7.90 - 8.08 (m, 4H), 8.14 (br s, 2H), 8.41 (d, J = 8.2 Hz, 2H) 8.51 - 8.66 (m, 2H); ^{13}C NMR (75 MHz, CD$_3$OD) δ = 45.7, 48.3, 127.3, 128.0, 128.2, 128.5, 132.4, 134.5, 137.8, 143.2, 150.2, 152.5, 181.9; IR (film) 1658, 1567, 1585, 1458, 1380, 1316, 698 cm^{-1}; HRMS (ESI$^+$) calcd. for C$_{24}$H$_{19}$N$_4$O [M+H]$^+$ 379.1553, found 379.1543.

Scheme 5. Synthesis of pyridine analog **8**
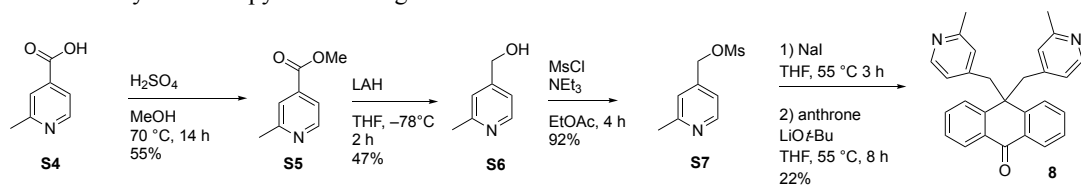
methyl 2-methylpyridine-4-carboxylate (S5). 2-Methylpyridine-4-carboxylic acid (0.200 g, 1.46 mmol) was added to an oven dried 20 mL vial. The vial was purged with nitrogen for 10 min. before anhydrous methanol (10 mL) was added. Concentrated H$_2$SO$_4$ (0.026 mL, 0.481 mmol) was added to the vial and the reaction was stirred in an oil bath heated to 70 °C for 14 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL), and the organic layer was washed with half sat. aq. sodium bicarbonate solution (10 mL). The aqueous layer was saturated with NaCl and extracted with EtOAc (4 x 10 mL). The combined organic layers were washed with brine (10 mL) and dried over sodium sulfate, then condensed down to yield the title compound as a clear oil (0.122 g, 56%). ^{1}H NMR showed the crude compound to be of sufficient purity to advance without further purification. This compound has been previously reported and characterized (CAS# 16830-24-3).^{1}HNMR (300 MHz, CDCl$_3$) δ = 2.59 (s, 3H), 3.90 (s, 3H), 7.59 (d, J = 5.0 Hz, 1H), 7.67 (s, 1H), 8.60 (d, J = 5.0 Hz, 1 H).

(2-methylpyridin-4-yl)methanol (S6). Ester **S5** (0.120 g, 0.778 mmol) was placed in an oven dried 20 ml vial. The vial was sealed under nitrogen, then anhydrous THF (6.0 mL) was added. LAH (0.031g, 0.817 mmol) was added to a separate oven dried 20 mL reaction vial. The vial was capped and purged with nitrogen before anhydrous THF (10.0 mL) was added. The solution was cooled to –78 °C using a dry ice and acetone bath, then the ester solution was added dropwise via syringe over 5 min. The reaction was stirred at –78 °C for 2 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was warmed to 0 °C, then was diluted with ether before a saturated sodium sulfate solution was added to quench the reaction. The mixture was stirred for 15 min. before being vacuum filtered and condensed to yield the title compound as a light yellow oil (0.041 g, 47%). ^{1}H NMR showed the crude compound to be of sufficient purity to advance without further purification. This compound has been previously reported and characterized (CAS# 105250-16-6). ^{1}H NMR (300 MHz, CD$_3$OD) δ = 2.51 (s, 3H), 4.63 (s, 3H) 7.21 (d, J = 5.3 Hz, 1H) 7.28 (s, 1H) 8.32 (d, J = 5.3 Hz, 1H).

(2-methylpyridin-4-yl)methyl methanesulfonate (S7). Alcohol **S6** (0.040 g, 0.325 mmol) was added to an oven dried 20 mL vial, followed by ethyl acetate (10 mL). Next, mesyl chloride (0.030 mL, 0.390 mmol) was added, followed by NEt$_3$ (0.054 mL, 0.390 mmol). The reaction was stirred for 4 h, after which time a sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was diluted with EtOAc (50 mL), and washed with water (10 mL) and brine before being condensed, yielding the title compound as a light yellow oil. The compound was used directly in the next step of the reaction without further purification or analysis.

10,10-bis[(2-methylpyridin-4-yl)methyl]-9,10-dihydroanthracen-9-one (8). Mesylate **S7** (0.011 g, 0.026 mmol) was added to an oven dried 20 mL reaction vial followed by NaI (0.005 g, 0.032 mmol). The vial was purged with nitrogen, then anhydrous THF (5.0 mL) was added. The reaction was stirred in an oil bath heated to 55 °C for 3 h. To a separate oven dried 8 mL reaction vial, anthrone (0.005 g, 0.026 mmol) and LiOt-Bu (0.008 g, 0.098 mmol) were added. The vial was purged with nitrogen, then anhydrous THF (3.0 mL) was added. The solution was sonicated until homogeneous before being added to the mesylate/iodide solution over 6 h via syringe pump. The reaction was then stirred for an additional 8 h at 55 °C. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with brine and diluted with EtOAc (75 mL). The organic layer was then washed with additional brine (3 x 50 mL). The organic layer was dried over sodium sulfate and condensed to give a light brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO$_2$ cartridge; 0–12% MeOH/DCM gradient) to give the title compound as a yellow semisolid (0.002 g, 22%). ^{1}H NMR (400 MHz, CD$_3$OD) δ = 2.05 (s, 6H), 3.85 (s, 4H), 6.11 (dd, J = 5.3, 1.4 Hz, 2H), 6.19 (s, 2H), 7.45 - 7.52 (m, 2H), 7.72 (d, J = 5.1 Hz, 2H), 7.84 - 7.94 (m, 2H), 8.00 (dd, J = 7.8, 1.6 Hz, 2H) 8.29 (d, J = 7.8 Hz, 2H).

10-[(3,5-difluoropyridin-4-yl)methyl]-9,10-dihydroanthracen-9-one (13). The mesylate **11** (0.099 g, 0.445 mmol) was added to an oven dried 50 mL round bottom flask containing NaI (0.051 g, 0.334 mmol). The flask was capped with a septum and purged with nitrogen before anhydrous THF (10 mL) was added via syringe. The flask was placed in an oil bath heated to 60 °C for 1 h. Meanwhile, anthrone (0.072 g, 0.371 mmol) and LiOt-Bu were added to an oven dried 25 mL round bottom flask. The flask was sealed with a septum and purged with nitrogen before anhydrous THF (10 mL) was added via syringe. After 30 min., the resulting anion was added by syringe to the mesylate solution at 60 °C under nitrogen, and the reaction was stirred for 12 h. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with brine and diluted with EtOAc (200 mL), and the aqueous layer was re-extracted with

EtAc (50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and condensed to give a brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO$_2$ cartridge, 0–50% EtOAc/hexanes gradient) to give the title compound as a tan semisolid (0.076 g, 63%). ^{1}H NMR (400 MHz, CDCl$_3$) δ = 3.13 (d, J = 7.0 Hz, 2H) 4.48 (t, J = 7.0 Hz, 1H) 7.16 - 7.24 (m, 2H) 7.50 (comp, 4 H) 8.20 (s, 2H) 8.26 (dd, J = 7.6, 1.4 Hz, 2H); ^{13}C NMR (75 MHz, (400 MHz, CDCl$_3$) δ = 36.1, 43.3, 122.7 (t, J = 16.8 Hz), 128.0, 128.2, 132.2, 133.0, 134.0 (dd, J = 25.3, 3.5 Hz), 143.2, 156.5 (d, J = 3.46 Hz), 159.9 (d, J = 3.46 Hz,) 184.4; ^{19}F NMR (376 MHz, CDCl$_3$) δ = −125.2; HRMS (ESI$^+$) calcd for C$_{20}$H$_{14}$F$_2$NO [M+H]$^+$ 322.1038, found 322.1045.

10-[(3,5-difluoropyridin-4-yl)methyl]-10-[(pyridazin-4-yl)methyl]-9,10-hydroanthracen-9-one (18). The alcohol **16** (0.015 g, 0.136 mmol) was added to an oven dried 20 mL reaction vial followed by DMAP (0.013 g, 0.102 mmol). The vial was sealed with a septum cap and anhydrous THF (5.0 mL) was added via syringe followed by NEt$_3$ (0.019 mL, 0.136 mmol) and MsCl (0.011 mL, 0.059 mmol). The reaction was stirred for 15 min. A sample aliquot was taken from the reaction, dissolved in 1 mL HPLC grade MeCN, and analyzed with LC-MS to confirm the completion of the reaction. The mesylate solution was filtered through a syringe filter (0.22 μm PTFE) under nitrogen into a capped, nitrogen flushed and oven dried 20 mL reaction vial containing NaI (0.010 g 0.065 mmol). The reaction was stirred at 60 °C for 30 min. Meanwhile, the monoalkylated anthrone **13** (0.028 g, 0.087 mmol) and LiOt-Bu (0.011 g, 0.131 mmol) were added to a separate oven dried 20 mL reaction vial. The vial was capped and purged with nitrogen before anhydrous THF (5.0 mL) was added via syringe. The solution was sonicated for 2 min. until homogeneous, then kept at room temperature for 30 min. The resulting anion solution was added to the mesylate/iodide solution at 60 °C via syringe over 2 min. The reaction was stirred for 30 min., then a sample aliquot was taken and dissolved in HPLC grade MeCN (1 mL) and analyzed with LC-MS to confirm the completion of the reaction. The reaction was quenched with brine, then diluted with EtOAc (150 mL). The organic layer was washed with brine, dried over sodium sulfate, and condensed to give a brown oil. The oil was dissolved in DCM and purified by flash chromatography (5 g SiO$_2$ cartridge, 0–12% MeOH/DCM gradient) to give the title compound as a light brown oil (0.023 g, 64%). ^{1}H NMR (300 MHz, CDCl$_3$) δ = 3.70 (s, 2H), 3.92 (s, 2H), 6.34 (dd, J = 5.6, 2.4 Hz, 1H), 7.50 (t, J = 7.5 Hz, 2 H), 7.75 (t, J = 7.6 Hz, 2H), 7.84 - 7.92 (m, 2H), 7.98 (s, 2H), 8.20 (dd, J = 7.9, 0.6 Hz, 2H), 8.41 (s, 1H) 8.59 (d, J = 5.3 Hz, 1H); ^{13}C NMR (75 MHz, CDCl$_3$) δ = 39.3, 44.5, 47.2, 120.5 (t, J = 16.7 Hz) 126.5, 127.3 (t, J = 1.73 Hz) 128.3, 128.6, 132.1, 133.8, 134.2 (dd, J = 25.5, 4.0 Hz), 135.9, 142.7, 150.5, 152.9, 156.0, 159.5, 182.1; ^{19}F NMR (376 MHz, CDCl$_3$) δ = −126.0; IR (film) 1662, 1601, 1421, 1284, 1060, 695 cm^{-1}; HRMS (ESI$^+$) calcd. for C$_{25}$H$_{18}$F$_2$N$_3$O [M+H]$^+$ 414.1412, found 414.1423.

Optimization of alkylation conditions to synthesize **6** (Table 1)

The mesylate **11** (0.025 g, 0.110 mmol) was added to an oven dried 8 mL reaction vial followed by NaI (varying amounts). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. The solution was placed in an oil bath heated to 50 °C for 3 h. Anthrone (**12**) (0.011 g, 0.055 mmol) was added to a separate oven dried 4 mL reaction vial, followed by base (variable amounts). The vial was capped and purged with nitrogen before anhydrous THF (2.0 mL) was added. After 1 h, the anthrone anion solution was added via dropwise over 2 min. via syringe to the mesylate/iodide solution at 50 °C under nitrogen, and the reaction was stirred for 12–16 h. The reaction was quenched with brine, then diluted with EtOAc (50 mL). The organic layer was washed with brine, dried over sodium sulfate, and condensed to give a brown oil. NMR yields were measured in CDCl$_3$ using pentachloroethane as an internal standard. Isolated yields were measured after the crude oil was dissolved in DCM and purified by flash chromatography (5 g SiO$_2$ cartridge, 0–12% MeOH/DCM gradient) to give the title compound (**6**).

4.2 Electrophysiology protocol

Electrophysiological patch-clamp recordings were all performed at room temperature (~21–23 ºC) using an Axopatch 200B amplifier coupled with an Axon Digidata 1550B data acquisition board (Molecular Devices Electrophysiology). Voltage responses were recorded using the whole-cell patch-clamp configuration in voltage clamp mode. To isolate KCNQ2/3 tail currents, voltage was held at –60 mV, before stepping to –20 mV. KCNQ2/3 tail currents were quantified as the current 5 ms after step back to –60 mV relative to the current at the end of the pulse. Patch pipettes had a resistance of 2 – 6 MΩ. Currents were sampled at 2 KHz with cell capacitance cancelled out and series resistances of < 10 MΩ compensated by 60%. The bath solution (Ringer's solution) contained 150 mM NaCl, 2.5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES, and 8 mM glucose, adjusted to pH 7.4 with NaOH. The internal solution used to fill the patch pipettes contained 175 mM KCl, 1 mM MgCl$_2$, 5 mM HEPES, 0.1 mM K$_4$BAPTA, 3 mM Na$_2$ATP, and 0.1 mM Na$_3$GTP, adjusted to pH 7.2 with KOH. The bath solution was perfused at 2 mL/minute, permitting solution exchange surrounding the recording cell with a time constant of 4 s.

4.3 Thallium flux assay protocol

For thallium flux assays of KCNQ2 and hERG, HEK-293 cells stably expressing either KCNQ2 or hERG were seeded in 384-well PureCoat amine plates at a density of 20,000 cells/well in 20 μL α-MEM containing 10% (v/v) Thermo fetal bovine serum and Corning glutagro. Cells were incubated overnight in a humidified 5% (v/v) CO$_2$ incubator at 37 ºC. On the day of the assay, test compounds were serially diluted over 10, 3-fold steps in DMSO and then further diluted to 2-fold over their final assay concentration (1:500) in Hanks Balanced Salt Solution, 20 mM HEPES pH 7.3. For loading of the thallium-sensitive fluorescent dye, the cell culture medium in the cell plate was replaced with 20 μL/well of a solution containing Hanks Balanced Salt Solution, 20 mM

HEPES pH 7.3, 0.03% (w/v) Pluronic F-127, 0.5% (v/v DMSO) and 1.3 µg/mL ION Biosciences Thallos AM. After a 1 hour incubation at 22 ℃, the dye-loaded cell plate was transferred to a WaveFront Panoptic kinetic imaging plate reader. Ten seconds of baseline data were collected at 1 Hz followed by addition 20 µL/well of test compounds prepared as described above. After 5 minutes of data collection at 1 Hz, 10 µL/well of a solution containing 142 mM potassium gluconate, 22.5 mM Tl_2SO_4, 1.3 mM $CaSO_4$, 0.9 mM $MgSO_4$, 5 mM glucose, and 20 mM HEPES pH 7.3 was added followed by an additional 2 minutes of data collection at 1 Hz. After data collection, fluorescence values were normalized (F/F_0) on a per well basis. Following normalization, values from 16 vehicle control wells were averaged and the resultant vehicle control wave was subtracted from each of the wells on the plate. Next, the initial slopes of the change in fluorescence were calculated for each of the wells. Potency values were obtained from fits of the slope values to a four-parameter logistic equation using IDBS XLfit add-in with Microsoft Excel. All data were obtained from triplicate wells on each day of assay and reported potency values are the average of three independent experiments.

4.4 Acid stability assay protocol

Compounds DMP-543 (3), ASA-8 (6), and JDP-107 (18) were added to separate 20 mL reaction vials. 1:1 (v/v) CH_3CN/0.2N aq. HCl was added to dilute the reactions to 3.0 mM. As an internal standard, furan-2-carboxylic acid (1.0 eq.) was added to each vial, and the reactions were placed in an oil bath heated at 60 °C for 36 h. At the desired time points, the reactions were directly analyzed by LC-MS. Degradation was measured using UV peak area of the original compounds at 254 nm, in comparison to furan-2-carboxylic acid as an internal standard.

ASSOCIATED CONTENT

Supplemental Material

^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra of select compounds.

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Author Contributions

Designed compounds: C.D. Synthesized and characterized compounds: J.D.P., A.A. Optimized reactions: J.D.P. Measured acid stabilities: J.D.P. Performed electrophysiology: O.L.V.-R., E.J.D. Performed ion flux assays: C.D.W. Performed compound profiling studies: E.D., L.A.A. Wrote the manuscript: C.D., J.D.P. Edited the manuscript and wrote the Supporting Info: C.D., J.D.P., C.D.W., E.J.D., L.A.A.

Notes

C.D.W. is an owner of WaveFront Biosciences and ION Biosciences, manufacturers of the Panoptic plate reader and thallium-sensitive dyes used for the thallium flux assays described in this manuscript.

ACKNOWLEDGMENT

ABBREVIATIONS

ACh, acetylcholine; clogP, calculated logarithm of ([*n*-octanol]/[water]) partition coefficient; CNS, central nervous system; CYP, cytochrome P450; DCM, dichloromethane; DMAP, 4-dimethylaminopyridine; hERG, human ether-à-go-go-related gene, a voltage-gated potassium channel equivalent to Kv11.1; KCNQ, voltage-gated potassium channels now identified as the family Kv7; Kv, voltage-gated potassium channel; LAH, lithium aluminum hydride; NIMH, National Institute of Mental Health; MPO, multi-parameter optimization; PCP, phencyclidine; Ms, methanesulfonyl or mesyl; PDSP, Psychoactive Drug Screening Program; pKa, negative logarithm of the acidity equilibrium constant; SAR, structure-activity relationship. THF, tetrahydrofuran; tPSA, topological polar surface area.

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