

Optimization of 8-Hydroxyquinolines as Inhibitors of Catechol O-Methyltransferase

Ingrid Buchler, Daniel Akuma, Vinh Q. Au, Gregory Carr, Pablo de León, Michael DePasquale, Glen Ernst, Yifang Huang, Martha Kimos, Anna Kolobova, Michael S Poslusney, Huijun Wei, Dominique Swinnen, Florian Montel, Florence Moureau, Emilie Jigorel, Monika-Sarah Schulze, Martyn Wood, and James C Barrow

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b01126 • Publication Date (Web): 01 Oct 2018

Downloaded from <http://pubs.acs.org> on October 1, 2018

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

Optimization of 8-Hydroxyquinolines as Inhibitors of Catechol *O*-Methyltransferase

Ingrid Buchler,[§] Daniel Akuma,[§] Vinh Au,[§] Gregory Carr,^{§,¶} Pablo de León,[§] Michael DePasquale,[§] Glen Ernst,[§] Yifang Huang,[§] Martha Kimos,[§] Anna Kolobova,[§] Michael Poslusney,[§] Huijun Wei,^{§,¶} Dominique Swinnen,[†] Florian Montel,[†] Florence Moureau,[†] Emilie Jigorel,[†] Monika-Sarah E.D. Schulze,^{||} Martyn Wood,[†] and James C. Barrow^{§,¶,*}

[§] Lieber Institute for Brain Development, 855 North Wolfe Street, Baltimore, MD 21205

[¶] Department of Pharmacology, Johns Hopkins University School of Medicine, 855 North Wolfe Street, Baltimore, MD 21287

[†] UCB Biopharma SPRL, B-1420 Braine-l'Alleud, Belgium

^{||} UCB Biopharma, 216 Bath Road, Slough SL1 3WE, United Kingdom

ABSTRACT A series of 8-hydroxy quinolines were identified as potent inhibitors of catechol *O*-methyltransferase (COMT) with selectivity for the membrane-bound form of the enzyme. Small substituents at the 7-position of the quinoline were found to increase metabolic stability without sacrificing potency. Compounds with good pharmacokinetics and brain penetration were identified and demonstrated in vivo modulation of dopamine metabolites in the brain. An X-ray co-crystal structure of compound **21** in the S-COMT active site shows chelation of the active site magnesium similar to catechol-based inhibitors. These compounds should prove useful for treatment of many neurological and psychiatric conditions associated with compromised cortical dopamine signaling.

INTRODUCTION

Proper functioning of dopamine signaling in the prefrontal cortex (PFC) is critical for a number of cognitive and behavioral processes that are impaired in a variety of conditions such as ADHD, obsessive-compulsive disorder, traumatic brain injury, and schizophrenia.¹ One approach towards selective modulation of dopamine signaling in the PFC is by inhibition of catechol *O*-methyltransferase (COMT), which is the predominant means of dopamine clearance in the PFC because of the lack of expression of synaptic dopamine transporters in this region,² in contrast to the striatum.

COMT is a magnesium-containing metalloenzyme that transfers a methyl group from the cofactor *S*-adenosylmethionine (SAM) to a number of endogenous and exogenous catechols such as dopamine, catecholestrogens, and dietary polyphenols. The COMT enzyme has two isoforms, designated *S*-COMT for the soluble form and MB-COMT for the membrane-bound form.³ The enzymatic domains of the two forms are identical, with MB-COMT having an extra 50 amino acids at the N-terminus for membrane anchoring that also influences enzymatic activity.⁴ The expression patterns of the two forms are different, with *S*-COMT predominating in the periphery and MB-COMT being more prevalent in the brain, although this differential expression is especially pronounced in humans compared to rodents.^{5 6} Since the goal is to achieve central COMT inhibition, identification of a compound with MB-COMT selectivity may be theoretically desirable.

Inhibitors of COMT are widely used for treatment of Parkinson's disease due to their role in inhibiting peripheral metabolism and thereby increasing levels of exogenously administered L-DOPA.⁷ Notably, the nitrocatechol scaffold has been exploited to provide the clinically used drugs tolcapone **1** and entacapone **2**, as well as the recently approved opicapone **3** (Figure 1).⁸ While they are effective in blocking peripheral COMT activity, entacapone and opicapone have negligible brain penetration, and tolcapone has low but measurable levels in the brain.⁹ Compounds with improved brain penetration have greater efficacy for the non-motor symptoms of Parkinson's disease,¹⁰ as well as have utility for other psychiatric and neurological conditions such as cognitive impairment in schizophrenia.¹¹ Despite the early clinical success achieved with tolcapone, the drug has been associated with hepatic toxicity.¹² Thus, the risk-benefit profile for tolcapone prevents its widespread deployment and new inhibitors of COMT are needed, especially those that are active in the brain as well as those derived from alternate pharmacophores to de-risk any toxicity arising from the nitro-catechol moiety.

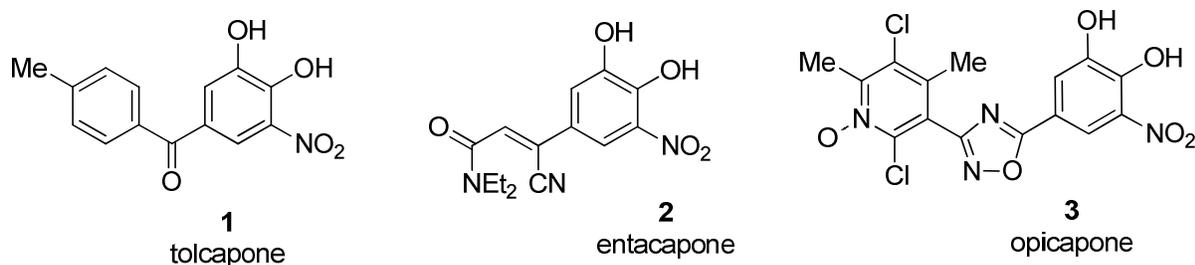


Figure 1. Clinically used COMT inhibitors

Soon after the discovery of COMT by Julius Axelrod in 1957,¹³ Ross and Haljasmaa investigated a number of compounds for their effects on COMT inhibition and identified 8-hydroxyquinoline (**4**, Figure 2) as a non-catechol-containing inhibitor.¹⁴ Later, Borchardt and colleagues further

1
2
3 explored this template with modest success,¹⁵ but the field subsequently shifted focus to
4
5 nitrocatechols given the exquisite potency and high ligand efficiency of the nitrocatechol
6
7 pharmacophore.^{7, 16} While **3** was able to overcome the rapid metabolic clearance associated with
8
9 tolcapone by taking advantage of a slow off rate that extends the pharmacodynamic action
10
11 beyond its short plasma half-life,¹⁷ nitrocatechols generally suffer from poor pharmacokinetics
12
13 and limited brain penetration. Herein, we describe further exploration of the 8-hydroxyquinoline
14
15 template and the discovery of potent inhibitors with excellent pharmacokinetics and brain
16
17 penetration.
18
19
20
21
22

23 **RESULTS AND DISCUSSION**

24
25
26
27
28 Borchardt's exploration of 5-substituted 8-hydroxyquinolines showed that sulfonic acid
29
30 (compound **5**, Figure 2) had similar COMT inhibition to other electron withdrawing groups
31
32 including chloro and nitro.¹⁵ In designing additional binding interactions for the 8-
33
34 hydroxyquinoline **4** (Figure 2) outside the metal chelating motif, modeling suggested substituents
35
36 on either the 5 or 6-position would overlay with nitrocatechol pharmacophore **1** (Figure 2). We
37
38 prepared both sulfone isomers **6** and **7** to guide future trajectories for exploration. While the 6-
39
40 substituted compound had a modest potency increase, the 5-position analog **7** appeared more
41
42 promising with an in vitro MB-COMT pIC₅₀ of 7.7.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

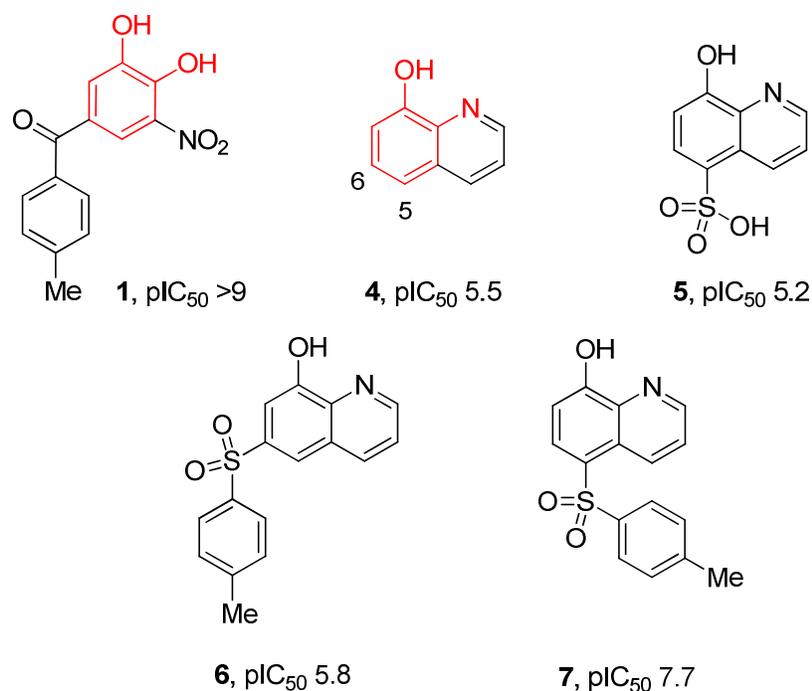
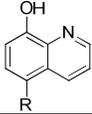
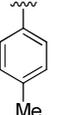
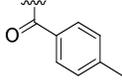
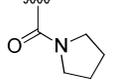
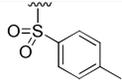
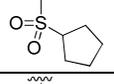
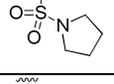
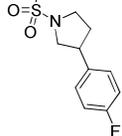
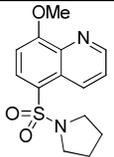


Figure 2. Evolution of the 8-hydroxyquinoline lead. pIC₅₀ values are reported for Human MB-COMT enzyme.

As shown in Table 1, a series of functional groups were appended at the 5-position, with sulfones **7** and **11** as well as sulfonamides **12** and **13** being preferable to ketone **9**, amide **10**, or direct aromatics like **8**. Additional sulfones and sulfonamides of varying sizes and polarity were prepared such as **13**; however, no meaningful increases in potency were achieved. A free hydroxyl is required for optimum hydrogen-bonding and magnesium coordination in the COMT active site, as compound **14** exhibited no appreciable inhibition of MB-COMT. Poor metabolic stability in rat hepatocytes was observed for representative compounds **12** and **13**, and the 8-hydroxyl moiety was suspected for phase II metabolism. Therefore, a series of sulfone and sulfonamide analogs were prepared that investigated substituents at the 7-position to explore steric and electronic effects on potency and metabolic stability.

Table 1. Potency and Hepatocyte Clearance of 5-substituted 8-hydroxyquinolines

			
#	R	Human MB-COMT ^a pIC ₅₀	Hepatocyte Clearance ^b (μL/min/10 ⁶ cells)
4	H	5.8±0.1	
8		6.6±0.1	
9		5.4±0.1	
10		<5	
7		7.7±0.1	
11		8.2±0.1	
12		8.1±0.3	177
13		8.5±0.2	255
14		<5	

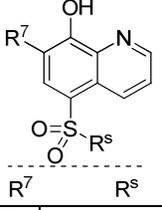
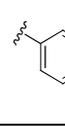
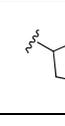
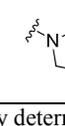
^a Potency determined by MTase GloTM Methyltransferase assay from membrane homogenate of recombinant human MB-COMT overexpressed in HEK cells. ^b Hepatocyte clearance in rat hepatocytes.

Expanding the SAR around 5-substituted sulfone and sulfonamide quinolines provided many potent and brain penetrant examples as described in Table 2. Both aryl and alkyl substitution were tolerated, as well as extension into larger and more diverse chemical space (data not shown,

1
2
3 see patent application WO2016123576 for additional examples).¹⁸ However, building larger and
4
5 more expansive sulfone and sulfonamides decreased ligand efficiency, increased lipophilicity
6
7 and diminished physicochemical properties thereby leaving small substituents with the best
8
9 combination of potency and physicochemical properties. The clearance profile of both
10
11 sulfonamides and sulfones is driven by phase II metabolism of the 8-hydroxy group; therefore,
12
13 screening in hepatocytes was used to capture the impact of metabolism in vitro. Poor metabolic
14
15 stability was observed with 7-unsubstituted 8-hydroxyquinolines as evident by the high
16
17 hepatocyte clearance for **15**, **16**, and **17** (Table 3). A tight correlation between rat hepatocytes
18
19 and in vivo clearance measured by PK studies was not evident; however, the hepatocyte screen
20
21 proved useful for triaging compounds with high clearance.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 2. Enzyme Potency of 5,7-substituted 8-hydroxyquinolines^a

#		Human MB-COMT ^a pIC ₅₀	Human S-COMT pIC ₅₀	Rat MB-COMT pIC ₅₀	Rat S-COMT pIC ₅₀	
15	H		7.3±0.3	5.8±0.4		
16	H		8.7±0.4	5.7±0.2		
17	H		7.2±0.1	5.9±0.5		
18	F		7.1±0.1	5.2±0.2		
19	Cl		6.7±0.03	<5		
20	F		8.4±0.7	5.8±0.1	>9	< 5
7	H		7.7±0.1	6.3±0.4		
21	F		8.2±0.3	6.3±0.4		
22	Cl		6.5±0.2	<5		
23	Br		5.9±0.4	<5		
24	CF ₃		<5	<5		
11	H			8.2±0.1	6.3±0.4	
25	F	8.7±0.3		5.6±0.03	>9	5.1
26	Cl	7.7±0.5		5.1±0.1	>9	< 5
27	CF ₃	<5		<5		
12	H		8.1±0.3	5.7±0.1		
28	F		8.4±0.6	5.8±0.4	>9	5.4
29	Cl		8.0±0.2	5.5±0.1		
30	Me		7.7±0.3	5.5±0.4		

^a Potency determined by MTase GloTM Methyltransferase assay from recombinant human or rat MB and S-COMT and are the average of three determinations.

Improvements in pharmacokinetic properties were observed when installing small groups at the 7-position of the quinoline, presumably by affecting the phase II metabolism of the 8-hydroxy moiety. Substituents to modulate the susceptibility of hydroxyl metabolism with respect to both steric and electronic effects were explored. As shown in Table 3, halogens generally showed improved in vitro and in vivo clearance while larger, more sterically bulky groups reduced MB-COMT potency (e.g. compound **23**). Strikingly, a CF₃ substituent at the 7-position (compound **24**) abolished MB-COMT inhibition. A small methyl substituent was tolerated (compound **30**) but proved to have no impact on clearance as measured by rat hepatocytes. Both fluoro and

chloro substitution yielded the best profiles with respect to metabolic stability, especially 7-chloro substituted compounds **19**, **26** and **29** which exhibited very low clearance and long half-life in rat PK studies (Table 3).

Table 3. ADME and Pharmacokinetic properties of 5,7-substituted 8-hydroxyquinolines

Example	Hepatocyte Stability (Rat) ($\mu\text{L}/\text{min}/10^6$ cells)	Rat PK ^b			
		%F	T _{1/2} (h)	Cl _p (ml/min/kg)	V _{ss} (L/kg)
11		24	0.18	26	1.8
12	177				
15	39				
16	228				
17	46				
18	12	66	3.5	13	3.8
19	4	23	8.9	0.31	0.23
20	15	100	4.5	12	3.5
21	36	34	0.26	27	1.2
22	78	45	0.31	25	0.67
25	19	32	7.7	0.62	0.41
26	18	37	11	0.3	0.3
28	21	73	2.8	11	2.2
29	14	33	8.6	1	0.8
30	103				

^b Rat pharmacokinetics after a 1mpk iv dose (n=3) and 3 or 10 mpk oral dose (n=3)

Brain penetration was initially assessed in mice by measuring total brain and plasma levels 5 minutes, 15 minutes, and 1 hour after an intraperitoneal (ip) 10 mg/kg dose in two mice at each timepoint (Supplementary Table 1 for all timepoints and Table 4 for the 1 hour timepoint). Brain penetration was generally rapid, so the 1 hour timepoint was chosen for compound comparison in Table 4. Additionally, free fraction in plasma and brain were measured by equilibrium dialysis. Early examples such as **15** and **16**, where hydrogen is in the 7-position exhibited low overall exposure consistent with poor pharmacokinetics. Compounds with either a 7-fluoro or chloro in both sulfone and sulfonamide moieties display good total plasma concentrations but modest brain exposures relative to total plasma. However, most examples in Table 2 have plasma protein binding approaching or greater than 99%, thereby limiting free drug concentrations. Additionally, the examples in Table 4 exhibited higher free fraction in rat brain

tissue as compared to plasma which has the expected effect of driving down total brain concentrations.¹⁹ Despite the protein/tissue binding differences, when correcting for free drug in both compartments most compounds had reasonable ratios of unbound plasma and unbound brain concentrations. The lower free brain drug concentrations relative to unbound plasma concentrations observed for compounds such as **20** and **22** are outliers to the trend and could not be explained by P-gp efflux as these compounds were not substrates for the transporter (Table 4) and have good apparent permeability (>100 nm/s, data not shown). Despite these two outliers, overall the observations described in Tables 4 and 5 reinforce the notion that optimization of the fraction unbound in the brain does not result in greater brain exposure, rather plasma free fraction is more correlative to drug levels in brain.¹⁹

Table 4. Mouse Brain Exposure of 5,7-substituted 8-hydroxyquinolines at one hour

Example	P-gp ER ^a	Plasma Protein Binding ^b	Brain Tissue Binding ^c	Mouse Brain Penetration ^d	
				Total Brain (ng/g)	Total Plasma (ng/ml)
11	NT	0.5 (r)	2.9	blq	173
12	NT	3.3 (m)	4.4	10.5	68
15	NT	6.9 (m)	10.3	11	250
16	NT	0.5 (m)	0.5	109	235
17	NT	1.4 (m)	NT	BLQ	3538
18	1.1	0.6 (r)	3.6	1094	2542
19	0.8	0.5 (r)	2.6	5655	16650
20	0.7	1.2 (r)	2.4	527	5720
21	NT	1.2 (r)	1.39	518	1744
22	1.1	0.84 (m)	0.5	1686	9780
25	0.5	0.2 (r)	4.2	1914	6160
26	NT	0.5 (r)	1.6	2792	14669
28	1.2	0.6 (r)	8	110	1819
29	0.4	0.5 (r)	3.4	1154	16250
30	NT	0.5 (m)	1.8	55	576

^a A:B/B:A efflux ratio from P-glycoprotein expressing MDCK cells. NT=not tested. ^b Percent free from either mouse (m) or rat (r) plasma as measured by equilibrium dialysis. ^c Percent free from rat brain tissue. ^d Total brain and total plasma concentrations 1 hour after ip dosing 10mg/kg to 2 mice.

Central nervous system inhibition of COMT produces measurable changes in dopamine metabolites dihydroxyphenyl acetic acid (DOPAC, increase) and homovanillic acid (HVA,

decrease). Assessment of these biomarkers of dopamine metabolism in rat CSF with accompanying measurement of inhibitor concentrations in plasma, brain homogenate, and CSF provides a rapid readout for compound brain penetration and activity. While the region of interest is the cortex, it has been demonstrated that CSF concentrations of HVA and DOPAC are reflective of total brain levels²⁰ and responsive to brain-penetrant COMT inhibitors.²¹

Table 5 shows the results from a single oral administration (10 mpk) of COMT inhibitors to rats (n=5-10, see Supplementary Table S2 for details). The animals were sacrificed 4 hours post dose where plasma, brain, and CSF were analyzed for dopamine metabolites and drug levels. Values for plasma protein binding and brain tissue binding (Table 4) were used to determine free plasma and brain levels. Changes in HVA and DOPAC in CSF as measured by LC-MS are shown as a difference between compound treated and vehicle treated controls. The positive control, tolcapone (15 mpk, ip injection), exhibited expected biomarker movement where DOPAC was increased and HVA decreased relative to control. Robust increases in DOPAC were observed for all five compounds tested. More modest decreases were observed for HVA, with only **25** and **28** showing a statistically significant difference compared to the vehicle treated group.

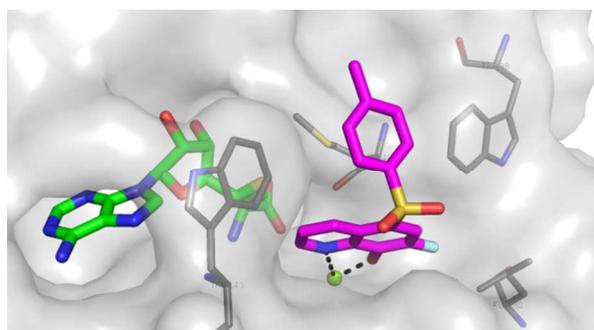
Example	Free Plasma (nM) ^a	Free Brain (nM) ^a	Free CSF (nM) ^b	CSF (nM)	CSF/ Free Plasma Ratio	CSF/ Free Brain Ratio	Free CSF/ Free Plasma Ratio	HVA (%) ^c	DOPAC (%) ^c
1	35	BLQ	NA	BLQ	N/A	N/A	N/A	28	304
20	48	7.7	59	127	2.6	16	1.2	86	173
25	19	12.8	139	321	16.5	25	7.3	66	218
26	32	5.6	NA	358	11	64	N/A	97	213
28	23	35	77	157	6.8	4.4	3.3	72	140

^a Free plasma and brain levels calculated using ratios listed in Table 4 on plasma and brain samples taken immediately after CSF sampling. Total concentrations are given in Supplementary Table 2. ^b Non-specific binding to CSF was determined by equilibrium dialysis using pooled rat CSF as described in reference 24. Compound **20** showed 53% binding, Compound **25** showed 57% binding, and Compound **28** showed 51% binding. ^c HVA and DOPAC changes (% of vehicle) 4 hours after 10mg/kg oral dose compared to vehicle (n=5-10 rats per group), except **1** (15mpk ip). BLQ=Below the limit of quantitation.

1
2
3 All four quinoline analogs produced good peripheral exposure and brain levels as predicted from
4 the mouse brain penetration studies. Measurable drug concentrations for all compounds in brain
5 homogenate extracts exceeded rat MB-COMT IC₅₀ measurements; however, this series has less
6 activity on S-COMT which may contribute to the modest changes in HVA relative to tolcapone.
7
8 Further studies with these compounds are in progress to obtain a more comprehensive
9 understanding of the effects of COMT inhibition by different classes of inhibitors. An
10 interesting additional observation is the lack of correlation between measured CSF levels and
11 calculated free plasma and free brain levels, which are expected to be similar for highly
12 permeable compounds that are not transporter substrates since the CSF has very low amounts of
13 protein compared to plasma.²² In addition to P-gp (Table 4), compound **28** was also examined
14 for susceptibility to BCRP and MRP2 transporters, as well as for inhibitory activity against P-gp,
15 BCRP, and MRP2. While compound **28** was not found to be a substrate or an inhibitor for these
16 three transporters (data not shown), that does not rule out susceptibility to other transporters
17 involved in blood-brain-CSF homeostasis.²³ This prompted measurement of free CSF
18 concentrations using equilibrium dialysis.²⁴ Compounds **20**, **25**, and **28** showed remarkably high
19 bound concentrations (51-57% bound @ 2 μM testing concentration) in rat CSF suggesting that
20 even though proteins like albumin are in CSF at less than 1% compared to plasma,²⁵ there exists
21 sufficient non-specific binding in CSF to effect the distribution of highly bound compounds.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

47 Confirmation of the proposed binding mode of the 8-hydroxyquinolines was confirmed by an X-
48 ray co-crystal structure with **21** and SAM in the rat S-COMT active site (Figure 3). As expected,
49 the hydroxyl and quinoline nitrogen make a bidentate interaction with the catalytic magnesium.
50
51 A free hydroxyl was observed to be required from the SAR studies (see Table 1, compound **14**),
52
53
54
55
56
57
58
59
60

1
2
3 and the structure reveals a tight hydrogen-bonding network around the hydroxyl similar to what
4 is seen with nitrocatechols.²⁶ The sulfone oxygens make no clear contacts with the protein, but
5 orient the aromatic ring between tryptophan-38 and tryptophan-143 and over the thioether
6 sidechain of methionine-40. The MB and S isoforms of COMT are identical except for an
7 additional 50 amino acids at the N-terminus of MB-COMT that are responsible for membrane
8 anchoring.²⁷ Without a structure of compound **21** bound to MB-COMT for comparison, it is
9 difficult to rationalize the selectivity for MB-COMT over S-COMT in this series, but it has been
10 proposed that residues 27-50 of MB-COMT lay back over the active site to provide additional
11 binding interactions.²⁸ The close proximity of the leucine-198 sidechain to the quinoline 7-
12 position explains the tolerance for only small substituents at the 7-position. As judged by the
13 potency differences seen in Table 1 between sulfone **7** and sulfonamide **12** versus ketone **9** and
14 amide **10**, other effects of the 5-substituent may be at play since those substituents should also be
15 able to “fit” in similar binding modes albeit at a torsional cost. Also notable is that there is no
16 electron density for the transferable methyl group of SAM, and the structure is modeled with
17 SAH despite high purity SAM used in the crystallization conditions.



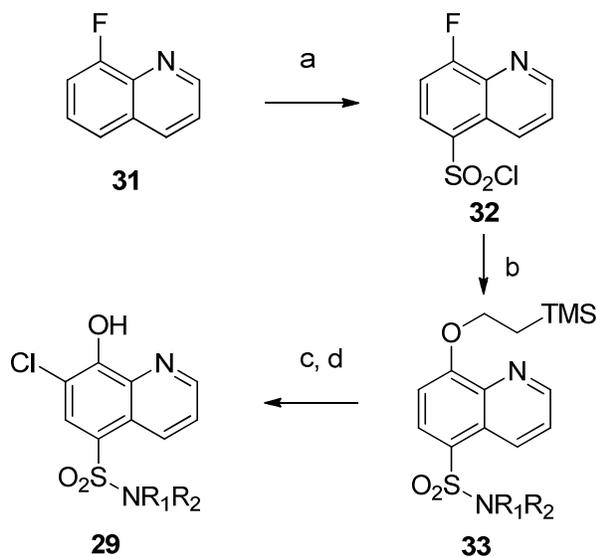
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49 **Figure 3.** Compound **21** (magenta) bound to the rat S-COMT active site (PDB 6GY1). The
50 hydroxyl and quinoline nitrogen make a bidentate interaction with the catalytic magnesium ion
51 (green sphere). The sulfone oxygens make no clear contacts with the protein, but orient the
52
53
54
55
56
57
58
59
60

1
2
3 aromatic ring between tryptophan-38 and tryptophan-143 and over the thioether sidechain of
4
5 methionine-40.
6
7
8
9

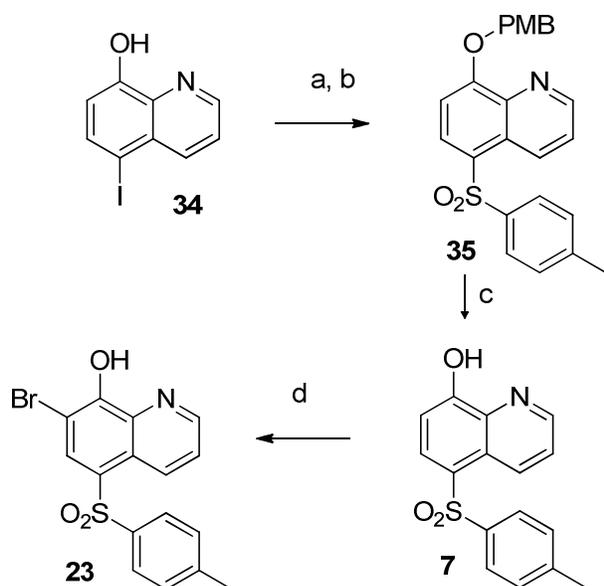
10 CHEMISTRY

11
12 Scheme 1 shows the preparation of unsubstituted and non-fluoro sulfonamides **12**, **13** and **16**,
13
14 beginning with chlorosulfonylation of 8-fluoroquinoline **31** followed by treatment of the desired
15
16 amine and quenching with sodium 2-(trimethylsilyl)ethan-1olate to displace the labile 8-fluoro
17
18 group to give **33**. Removal of the TMSE protecting group followed by treatment with N-chloro
19
20 succinimide (NCS) gave the desired sulfonamide **29**. The corresponding sulfones **7** and **23** were
21
22 prepared according to Scheme 2 by PMB protection of 5-iodo-8-hydroxyquinoline **35** followed
23
24 by copper (I) mediated coupling with 4-methylbenzenesulfinate to install the sulfone group.²⁹
25
26 The protecting group was removed with TFA at ambient temperature to provide compound **7**.
27
28 Bromination of **7** was achieved with NBS in chloroform to give example **23**. Additional 7-
29
30 chloro sulfone analogs were prepared by chlorination with NCS followed by benzylating 5-
31
32 chloro sulfone analogs were prepared by chlorination with NCS followed by benzylating 5-
33
34 bromo-8-hydroxyquinoline **36** (Scheme 3). Palladium catalyzed cross coupling of thiols
35
36 produced thioethers **35**.³⁰ The desired thioethers could be prepared in either one step
37
38 (compounds **22** and **26**) or by use of an intermediary TMS-thioethane (example **19**). Oxidation
39
40 of the sulfur with mCPBA gave sulfones with minimal quinoline N-oxide formation. Acid-
41
42 catalyzed deprotection of the benzyl group provided the desired analogs **39**. Incorporation of a
43
44 fluorine at the 7-position of the quinoline was accomplished as described in Scheme 4. A Skraup
45
46 reaction³¹ was used to prepare the 7-fluoro-8-hydroxyquinoline **41** which could be either
47
48 chlorosulfonylated and quenched with amines to give sulfonamide **28**, or iodinated and protected
49
50 to give key intermediate **42**. Palladium catalyzed coupling of thiols followed by oxidation and
51
52
53
54
55
56
57
58
59
60

deprotection gave the desired sulfones **44**. Other quinolines shown in Table 1 could be prepared as shown in Scheme 5, via direct Friedel-Crafts acylation to give ketone **9**, or introduction of the 8-hydroxyl as a protected benzyl by S_NAr on 8-fluoroquinolines **46** or **48** which led to compounds **10** and **6** respectively.

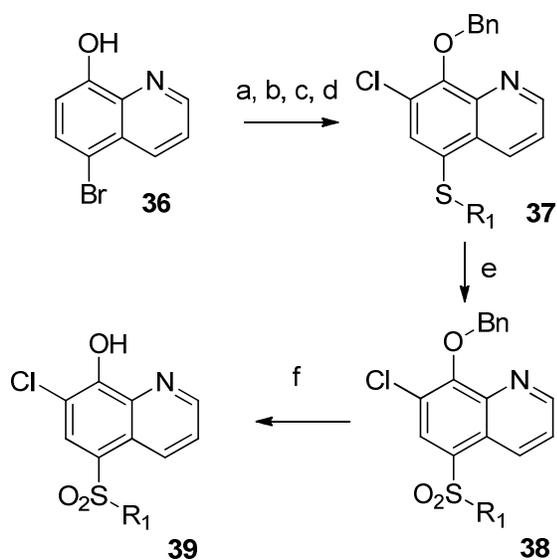


Scheme 1. General synthetic procedures for sulfonamides **12**, **13**, **16**, **29**. Reagents and conditions: (a) chlorosulfonic acid, 100 °C, 71%; (b) DIPEA, amine, THF then NaH, 2-(trimethylsilyl)ethanol; (c) CsF, DMF, 60 °C, 84% or TBAF, THF, 34%; (d) NCS, $CHCl_3$, 60°C, 24%.



23
24
25
26
27
28
29

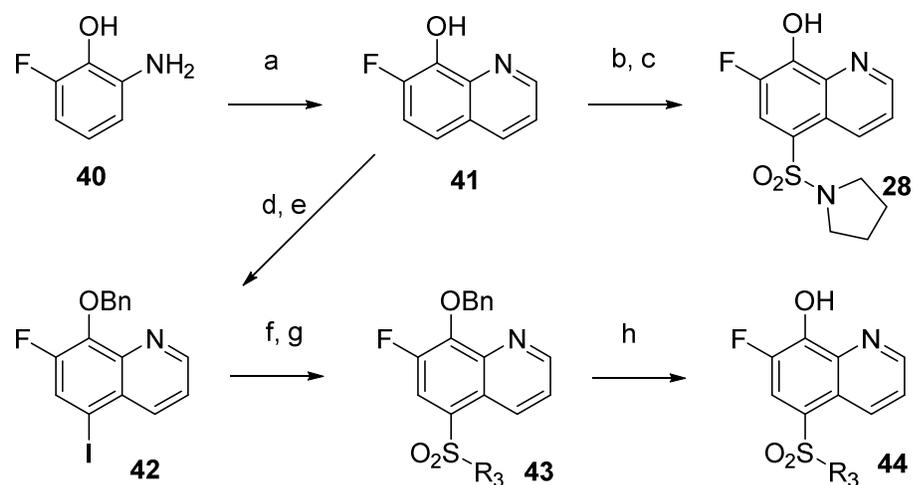
Scheme 2. Synthetic protocol for **7** and **23**. Reagents and conditions: (a) K_2CO_3 , PMB-Cl, ACN, 22%; (b) CuI, DMSO, 4-methylbenzenesulfinate, 90 °C, 22%; (c) TFA, rt, 92%; (d) NBS, $CHCl_3$, 85%.



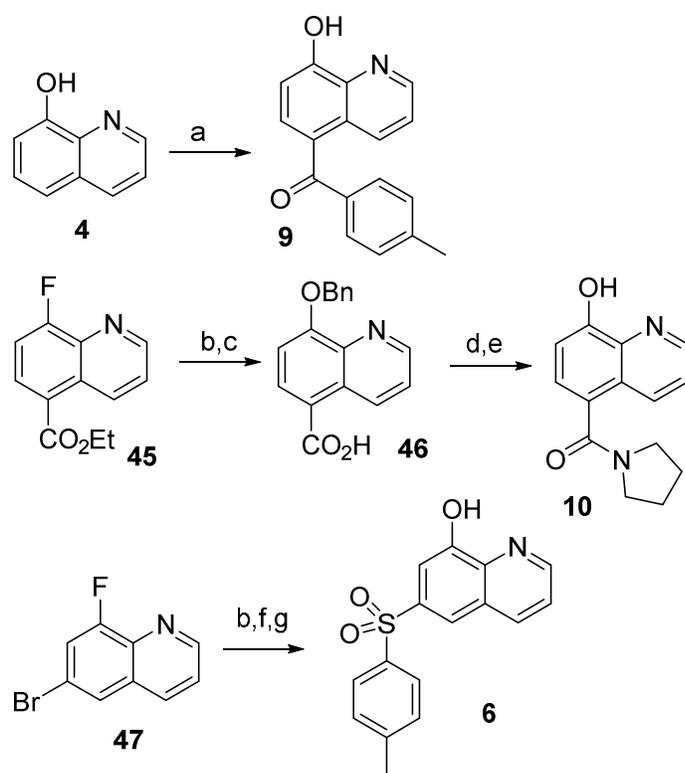
48
49
50
51
52
53
54
55
56
57
58
59
60

Scheme 3. General synthetic procedures for **19**, **22**, and **26**. Reagents and conditions: (a) NCS, $CHCl_3$, 78%; (b) K_2CO_3 , BnBr, ACN, 89%; (c) 2-trimethylsilylethanethiol (example **19**) or R_1SH (examples **22** and **26**), Xantphos, Cs_2CO_3 , $Pd_2(dba)_3$, dioxane, 100 °C, 93% example **19**;

81% example **22**, 93% example **26**; (d) for example **19**, 4-fluoriodobenzene, TBAF, Xantphos, Cs₂CO₃, Pd₂(dba)₃, dioxane, 90 °C, 93%; (e) mCPBA, DCM; (f) 6M aqueous HCl, 100 °C.



Scheme 4. General synthetic protocol for **18**, **20**, **21**, **25**, and **28**. Reagents and conditions: (a) glycerol, sodium-3-nitrobenzenesulfonate, 70% sulfuric acid, 140 °C, 56%; (b) chlorosulfonic acid, CHCl₃, 100 °C, 40%; (c) triethylamine, pyrrolidine, DCM, 0 °C, 98%; (d) NIS, CHCl₃, 40 °C, 92%; (e) NaH, BnBr, DMF, 73%; (f) thiol, Xantphos, Cs₂CO₃, Pd₂(dba)₃, dioxane, 90 °C; (g) mCPBA, DCM; (h) 6M aqueous HCl, 100 °C.



Scheme 5. Synthetic protocols for ketone **9**, amide **10**, and sulfone **6**. a) AlCl_3 , 4-methylbenzoyl chloride, toluene, $110\text{ }^\circ\text{C}$, 5%; b) NaH , BnOH , THF; c) NaOH , THF/ H_2O , 63%; d) 1-Chloro-N,N,2-trimethyl-1-propenylamine, DCM then pyrrolidine; e) HBr , acetic acid, 63% over 2 steps; f) Cu(I)I , K_2CO_3 , DMEN, DMSO, sodium 4-methylbenzenesulfinate, 46%; g) 6M HCl , 87%.

CONCLUSIONS

A series of 8-hydroxyquinolines were prepared and evaluated as COMT inhibitors, and several potent MB-COMT inhibitors were identified that demonstrate selectivity over S-COMT. An X-ray co-crystal structure highlights the important chelation of the active site magnesium; however, the interactions responsible for the observed MB-COMT selectivity are not apparent in the S-

1
2
3 COMT structure. Small substituents at the 7-position were found to increase metabolic stability
4 without sacrificing potency, and compounds with good pharmacokinetics such as **25** and **28** were
5 identified. This series of compounds generally has good brain penetration, although high plasma
6 protein binding results in high exposures required for efficacy in a rat biomarker assay of COMT
7 activity. Further in vivo characterization of these compounds on central nervous system
8 dopamine metabolism will be reported in due course.
9
10
11
12
13
14
15
16
17
18

19 **EXPERIMENTAL SECTION**

20
21
22
23

24 **General Procedures.** All commercially available reagents and solvents were used without
25 further purification unless otherwise stated. Automated flash chromatography was performed on
26 an ISCO CombiFlash RfTM or Biotage IsoleraTM using Biotage Flash cartridges with peak
27 detection at 254 nm. Reverse phase purification was accomplished using a Gilson 215 liquid
28 handler equipped with a Phenomenex C18 column (150 x 20 mm I.D., S-5 μ m). Peak collection
29 was triggered by UV detection at 214 or 254 nm. ¹H NMR spectra were recorded on a Bruker
30 400 instrument operating at 400 MHz with tetramethylsilane or residual protonated solvent used
31 as a reference. Analytical LC-MS was performed using Agilent 1260 equipped with autosampler
32 (Agilent Poroshell 120 C18 column (50 x 3.0 mm I.D., 2.7 μ m); 0.05% TFA in water/acetonitrile
33 gradient; UV detection at 215 and 254 nm) and electrospray ionization. All final compounds
34 showed purity greater than 95% at 215 and 254 nm using this method.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

51 **6-Tosylquinolin-8-ol (6).** *Step 1.* To a solution of benzyl alcohol (1.1 mL, 10.8 mmol)
52 in THF (30 mL) was added sodium hydride (0.44 g, 10.8 mmol) portionwise over 5 min. The
53
54
55
56
57
58
59
60

1
2
3 resulting mixture was allowed to stir for 30 min, then a solution of 6-bromo-8-fluoro-quinoline
4 **47** (1.6 g, 7.2 mmol) in THF (15 mL) was added dropwise over 5 min. The contents were stirred
5
6 at room temperature for 4 days (for convenience). The reaction mixture (which had become
7
8 gelatinous) was taken up in EtOAc and washed with water (4 X), brine (1 X), dried over MgSO₄,
9
10 filtered, and the solvent removed in vacuo. The desired product crystallised upon standing and
11
12 was washed with ethyl acetate then air-dried to give 0.53 g of pure desired product. The filtrate
13
14 was concentrated and purified by automated normal-phase chromatography (0-40%
15
16 EtOAc/hexanes, 50 g silica gel cartridge). The product-containing fractions were combined and
17
18 the solvent removed in vacuo to give a white solid, which when combined with the crystallized
19
20 material to provide a total of 8-benzyloxy-6-bromo-quinoline **48** (1.3 g, 57% yield) as a white
21
22 solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.88 (dd, *J* = 4.04, 1.77 Hz, 1 H) 8.31 (dd, *J* =
23
24 8.34, 1.77 Hz, 1 H) 7.83 (d, *J* = 2.02 Hz, 1 H) 7.60 (dd, *J* = 8.34, 4.04 Hz, 1 H) 7.53 - 7.57 (m, 2
25
26 H) 7.42 - 7.47 (m, 3 H) 7.35 - 7.41 (m, 1 H) 5.34 (s, 2 H). MS (ES+) *m/z* 314.0 [M+H]⁺.
27
28
29
30
31
32

33 *Step 2.* A mixture of copper (I) iodide (28 mg, 0.15 mmol), 8-benzyloxy-6-bromo-
34
35 quinoline **48** (100 mg, 0.32 mmol), sodium 4-methylbenzenesulfinate (170 mg, 0.95 mmol) and
36
37 potassium carbonate (88 mg, 0.64 mmol) and N,N'-dimethylethylenediamine (6.8 μL, 0.06
38
39 mmol) in DMSO (3 mL) was heated to 90°C for 60 min under microwave heating. The contents
40
41 were diluted with EtOAc, washed with water (3 X), brine, dried over MgSO₄, filtered and the
42
43 solvent removed in vacuo to give a yellow residue. This material was purified by automated
44
45 normal-phase chromatography (0-100% EtOAc/hexanes, 4 g silica gel cartridge). The product-
46
47 containing fractions were combined and the solvent removed in vacuo to give 8-benzyloxy-6-(p-
48
49 tolylsulfonyl)quinoline **49** (57 mg, 46% yield) as a beige solid. ¹H NMR (400 MHz, CDCl₃) δ
50
51 ppm 9.07 (dd, *J* = 4.17, 1.64 Hz, 1 H) 8.22 - 8.27 (m, 1 H) 8.07 (d, *J* = 1.77 Hz, 1 H) 7.66 - 7.71
52
53
54
55
56
57
58
59
60

(m, 2 H) 7.53 - 7.58 (m, 1 H) 7.47 - 7.52 (m, 2 H) 7.41 (d, $J = 2.02$ Hz, 1 H) 7.31 - 7.39 (m, 3 H) 7.22 (d, $J = 8.08$ Hz, 2 H) 5.48 (s, 2 H) 2.39 (s, 3 H). MS (ES+) m/z 390.0 $[M+H]^+$.

Step 3. 8-benzyloxy-6-(p-tolylsulfonyl)quinoline **49** (55 mg, 0.14 mmol) and 6 M hydrochloric acid (0.64 mL, 3.8 mmol) were stirred at 100°C for 4 h, then allowed to cool to room temperature. The solid was collected by filtration, washed with water and dried under vacuum to give 6-(p-tolylsulfonyl)quinolin-8-ol **6** (36 mg, 87% yield) as a beige solid. ^1H NMR (400 MHz, DMSO- d_6) δ ppm 9.05 (dd, $J = 4.42, 1.64$ Hz, 1 H) 8.75 (d, $J = 8.34$ Hz, 1 H) 8.24 (d, $J = 2.02$ Hz, 1 H) 7.88 (d, $J = 8.34$ Hz, 2 H) 7.81 (dd, $J = 8.34, 4.29$ Hz, 1 H) 7.45 (dd, $J = 5.05, 3.03$ Hz, 3 H) 2.37 (s, 3 H). MS (ES+) m/z 300.0 $[M+H]^+$.

5-Tosylquinolin-8-ol (7). *Step 1.* To a stirring solution of 5-iodoquinolin-8-ol **34** (4.1 g, 15.3 mmol) in acetonitrile (100 mL) was added potassium carbonate (4.2 g, 30.8 mmol). The suspension was stirred at room temperature for 30 minutes. 4-Methoxybenzyl chloride (2.5 mL, 18.4 mmol) was added and the resultant suspension was heated at 80°C for 4 hours. After cooling to room temperature, the suspension was filtered, the filter cake washed with ethyl acetate and filtrate was concentrated to a residue. The residue was purified by automated normal-phase chromatography and eluted with ethyl acetate/hexanes (0-80%) to give 8-((4-methoxybenzyl)oxy)-quinoline **50** as an off-white solid (1.4 g, 22 % yield). ^1H NMR (400 MHz, DMSO- d_6) δ ppm 8.86 (dd, $J = 4.04, 1.52$ Hz, 1 H) 8.29 (dd, $J = 8.46, 1.64$ Hz, 1 H) 8.06 -8.11 (m, 1 H) 7.67 (dd, $J = 8.59, 4.29$ Hz, 1 H) 7.43 - 7.49 (m, 2 H) 7.15 (d, $J = 8.34$ Hz, 1 H) 6.95 - 7.01 (m, 2 H). MS (ES+) m/z 392.0 $[M+H]^+$.

Step 2. To a stirring solution of 5-iodo-8-((4-methoxybenzyl)oxy)quinoline **50** (0.20 g, 0.51 mmol) in DMSO (1.5 mL), copper (I) iodide (10 mg, 0.051 mmol), sodium (S)-pyrrolidine-

1
2
3 2-carboxylate (14 mg, 0.10 mmol) and sodium 4-methylbenzenesulfinate (109 mg, 0.61 mmol)
4
5 were added. The solution was heated at 90°C for 24 hrs. Water (2 mL) was added and precipitate
6
7 isolated by filtration. The crude material was purified by automated normal-phase
8
9 chromatography, using ethyl acetate/hexanes (0-80%) as an eluent to give 8-((4-
10
11 methoxybenzyl)oxy)-5-tosylquinoline **35** as an off-white solid (47 mg, 22% yield). ¹H NMR
12
13 (400 MHz, CDCl₃) δ ppm 9.00 (s, 2 H) 8.42 (d, *J* = 8.34 Hz, 1 H) 7.81 (d, *J* = 8.34 Hz, 2 H)
14
15 7.53 (dd, *J* = 8.84, 4.29 Hz, 1 H) 7.45 (d, *J* = 8.59 Hz, 2 H) 7.24 - 7.31 (m, 4 H) 7.16 (d, *J* =
16
17 8.59 Hz, 1 H) 6.88 - 6.99 (m, 2 H) 5.45 (s, 2 H) 3.79 - 3.86 (m, 3 H). MS (ES+) *m/z* 420.0
18
19 [M+H]⁺.
20
21
22
23

24 *Step 3.* 8-((4-Methoxybenzyl)oxy)-5-tosylquinoline **35** (47 mg, 0.11 mmol) was
25
26 dissolved in TFA (0.86 mL, 11.2 mmol) and stirred at room temperature for 1 hour. The solution
27
28 was concentrated. The residue was purified by recrystallization in ethyl acetate/hexanes to give
29
30 5-tosylquinolin-8-ol **7** as an off-white solid (30 mg, 92% yield). ¹H NMR (400 MHz, DMSO-
31
32 *d*₆) δ ppm 8.94 (dd, *J* = 4.17, 1.64 Hz, 1 H) 8.89 (dd, *J* = 8.72, 1.64 Hz, 1 H) 8.39 (d, *J* = 8.34
33
34 Hz, 1 H) 7.80 - 7.86 (m, 2H) 7.73 (dd, *J* = 8.72, 4.17 Hz, 1 H) 7.35 - 7.41 (m, 2 H) 7.27 (d, *J* =
35
36 8.34 Hz, 1 H) 2.32 (s, 3 H). MS (ES+) *m/z* 300.0 [M+H]⁺.
37
38
39
40
41

42 **5-(p-Tolyl)quinoline-8-ol (8).** *Step 1.* To a solution of 5-bromoquinolin-8-ol **36**
43
44 (5 g, 22.3 mmol) in DMF (40 mL) and THF (20 mL) was added sodium hydride (1.1 g, 26.7
45
46 mmol). After 15 minutes benzyl bromide (3.1 mL, 26.7 mmol) was added slowly dropwise. The
47
48 reaction was maintained at ambient temperature overnight. The reaction was quenched by
49
50 addition of water, solids formed and were collected by filtration to yield 7.3 g (assume
51
52 quantitative yield) of 8-benzyloxy-5-bromo-quinoline **51**. ¹H NMR (400 MHz, DMSO-*d*₆) δ
53
54
55
56
57
58
59
60

1
2
3 ppm 8.95 (br. s., 1 H) 8.47 (d, $J = 8.08$ Hz, 1 H) 7.88 (d, $J = 8.34$ Hz, 1 H) 7.73 (br. s., 1 H) 7.55
4
5 (br. s., 2 H) 7.41 (dd, $J = 17.68, 6.82$ Hz, 3 H) 7.28 (d, $J = 8.08$ Hz, 1 H) 5.34 (br. s., 2 H). MS
6
7 (ES+) m/z 314.0 $[M+H]^+$.
8
9

10 *Step 2.* A vial was charged with 8-benzyloxy-5-bromo-quinoline **51** (150 mg, 0.48
11 mmol), Pd(PPh₃)₄ (55 mg, 0.05 mmol), 4-methylphenylboronic acid (77 mg, 0.57
12 mmol), NaHCO₃ (0.48 mL, 0.95 mmol) 2M aqueous solution in 1,4-dioxane (5 mL). The vial
13
14 was sparged with nitrogen for 5 minutes and heated at 90°C overnight. The reaction was
15
16 concentrated to a residue, resultant residue partitioned between water and ethyl acetate, layers
17
18 separated, aqueous extracted (2 X 2 mL), organics passed through a phase separator tube and
19
20 solution concentrated to an oil. Purification was accomplished by automated silica gel
21
22 chromatography (12 g pre-packed silica cartridge) eluting with 0-100% ethyl acetate/heptane to
23
24 provide 8-benzyloxy-5-(p-tolyl)quinoline **52** as a yellow glass (150 mg, 97% yield). ¹H NMR
25
26 (400 MHz, DMSO-*d*₆) δ ppm 2.40 (s, 3 H) 5.36 (s, 2 H) 7.33 - 7.40 (m, 4 H) 7.40 - 7.48 (m, 4 H)
27
28 7.51 - 7.61 (m, 4 H) 8.17 (dd, $J=8.59, 1.52$ Hz, 1 H) 8.89 (dd, $J=4.04, 1.52$ Hz, 1 H). MS (ES+)
29
30 m/z 326.0 $[M+H]^+$.
31
32
33
34
35
36
37

38 *Step 3.* A solution of 8-benzyloxy-5-(p-tolyl)quinoline **52** (150 mg, 0.46 mmol)
39 dissolved in 1,4-dioxane (2 mL) and 6N aqueous hydrochloric acid (3 mL, 18 mmol) was heated
40
41 at 100°C for 4 hours. The material was concentrated to a residue and purified by automated
42
43 reversed phase HPLC to yield 85 mg (52%) of 5-(p-tolyl)quinoline-8-ol **8** as a TFA salt. ¹H
44
45 NMR (400 MHz, DMSO-*d*₆) δ ppm 2.40 (s, 3 H) 7.30 (d, $J=8.08$ Hz, 1 H) 7.33 - 7.37 (m, 4 H)
46
47 7.51 (d, $J=8.08$ Hz, 1 H) 7.73 (dd, $J=8.59, 4.55$ Hz, 1 H) 8.48 (d, $J=8.34$ Hz, 1 H) 8.98 (dd,
48
49 $J=4.55, 1.52$ Hz, 1 H). MS (ES+) m/z 236.2 $[M+H]^+$.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **(8-Hydroxyquinolin-5-yl)(p-tolyl)methanone (9)**. To a mixture of 8-
4 hydroxyquinoline **4** (250 mg, 1.7 mmol) dissolved in toluene (3 mL) was added 4-methylbenzoyl
5 chloride (0.23 mL, 1.7 mmol). The resultant solution was cooled to 0°C and aluminum chloride
6 (574 mg, 4.3 mmol) was added in 2 portions. The resultant mixture was allowed to warm to
7 ambient temperature for 2 hours. The material was quenched by pouring over ice and 1M HCl
8 aqueous solution. The acidic solution was extracted with ethyl acetate (2 X 10 mL). The organics
9 were combined, dried over sodium sulfate and concentrated to a residue. Purification was
10 accomplished by automated normal-phase chromatography using 0-5%
11 methanol/dichloromethane as an eluent to provide 24 mg (5% yield) of title compound **9**. ¹H
12 NMR (400 MHz, DMSO-d₆) δ ppm 8.89 (br. s., 1 H) 8.53 (d, *J* = 7.83 Hz, 1 H) 8.11 (d, *J* = 8.08
13 Hz, 2 H) 7.99 (d, *J* = 6.82 Hz, 1 H) 7.68 - 7.76 (m, 2 H) 7.64 (d, *J* = 4.55 Hz, 1 H) 7.46 (d, *J* =
14 7.58 Hz, 2 H) 2.45 (s, 3 H). MS (ES+) *m/z* 264.1 [M+H]⁺.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33 **(8-Hydroxyquinolin-5-yl)(pyrrolidin-1-yl)methanone (10)**. *Step 1*. To a solution of
34 ethyl 8-hydroxyquinoline-5-carboxylate **45** (270 mg, 1.2 mmol) in dry DMF (10 mL) was added
35 sodium hydride (60 mg, 1.4 mmol) at ambient temperature. After 5 minutes benzyl bromide
36 (0.18 mL, 1.4 mmol) was added in a dropwise manner. The reaction was allowed to stir at
37 ambient temperature. After 15 minutes the reaction was quenched by addition of ice-water. The
38 aqueous mixture was extracted (2 X 20 mL) ethyl acetate, organic washes combined, dried over
39 sodium sulfate and concentrated to a residue. Purification was accomplished automated normal-
40 phase chromatography (40 g silica gel cartridge) eluting 0-100% ethyl acetate/hexanes to provide
41 259 mg as a 1:1 mixture of ethyl-8-benzyloxyquinoline-5-carboxylate **53** and 8-
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

benzyloxyquinoline. The mixture was taken on in the next step without additional purification.

MS (ES+) m/z 308.0 [M+H]⁺.

Step 2. To a solution of ethyl-8-benzyloxyquinoline-5-carboxylate **53** mixture (250 mg, 0.81 mmol) in THF (10 mL) was added water (10 mL). The mixture was allowed to stir at 40°C overnight. The pH was adjusted to ~4-5 by addition of 4.5 M aqueous hydrochloric acid. Solids formed and collected by filtration, washed with water and dried in a vacuum oven to yield 8-benzyloxyquinoline-5-carboxylate **46** (144 mg, 63% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 12.99 (br. s., 1 H) 9.42 (dd, *J* = 8.84, 1.77 Hz, 1 H) 8.91 (dd, *J* = 4.04, 1.52 Hz, 1 H) 8.26 - 8.31 (m, 1 H) 7.68 (dd, *J* = 8.59, 4.04 Hz, 1 H) 7.57 (d, *J* = 6.82 Hz, 2 H) 7.42 - 7.48 (m, 2 H) 7.34 - 7.41 (m, 2 H) 5.39 (s, 2 H). MS (ES+) m/z 280.0 [M+H]⁺.

Step 3. To a slurry of 8-benzyloxyquinoline-5-carboxylate **46** (61 mg, 0.22 mmol) in dry dichloroethane (2 mL) was added Ghosez reagent (0.29 mL, 2.1 mmol) at ambient temperature. To drive the reaction into solution additional Ghosez reagent was added (totaling 0.7 mL). After 1 hour, pyrrolidine (0.54 mL, 6.5 mmol) was added. The reaction was stirred at ambient temperature for 1 hour then diluted with dichloromethane and extracted (1 X 3 mL) water. The material was passed through a phase separator tube and organic layer concentrated to a residue. Purification was accomplished by automated normal-phase chromatography using 0-10% methanol/dichloromethane as an eluent. The oily (8-benzyloxy-5-quinolyl)-pyrrolidin-1-yl-methanone solidified upon standing (102 mg). The crude material was taken on without additional purification and or characterization.

A mixture of (8-benzyloxy-5-quinolyl)-pyrrolidin-1-yl-methanone (83 mg, 0.29 mmol) dissolved in acetic acid (0.5 mL) was added hydrobromic acid (0.5 mL). The resultant mixture was heated to 100°C for 3 hours. The material was cooled to ambient temperature and

1
2
3 neutralized with 6 M sodium hydroxide. The aqueous solution was extracted (3 X 2 mL)
4
5 dichloromethane, organics combined, filtered through a phase separator tube and concentrated to
6
7 a residue. Purification was accomplished by automated normal-phase chromatography using 0-
8
9 10% methanol/dichloromethane as an eluent. The impure material was concentrated to a residue
10
11 then purified by automated reversed-phase chromatography eluting 5-95% acetonitrile/water,
12
13 0.05% trifluoroacetic acid as modifier. A yellow foam of 8-hydroxyquinolin-5-yl(pyrrolidin-1-
14
15 yl)methanone **10** (57 mg, 43% yield) was obtained. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.95
16
17 (dd, *J* = 4.17, 1.39 Hz, 1 H) 8.47 (d, *J* = 8.59 Hz, 1 H) 7.73 (dd, *J* = 8.34, 4.55 Hz, 1 H) 7.59 (d,
18
19 *J* = 8.08 Hz, 1 H) 7.17 (d, *J* = 8.08 Hz, 1 H) 3.58 (t, *J* = 6.82 Hz, 2 H) 3.16 (t, *J* = 6.44 Hz, 2 H)
20
21 1.86 - 1.97 (m, 2 H) 1.73 - 1.84 (m, 2 H). MS (ES+) *m/z* 243.0 [M+H]⁺.
22
23
24
25
26
27

28 **5-(Cyclopentylsulfonyl)quinoline-8-ol (11).** *Step 1.* 8-Hydroxyquinoline-5-sulfonic
29
30 acid hydrate (10 g, 41.1 mmol) was dissolved in sodium hydroxide solution (1 N, 103 mL, 103
31
32 mmol) and heated to 75°C. A solution of benzyl chloride (10.4 ml, 90 mmol) in THF (100 mL)
33
34 was added and resultant mixture maintained at 75°C overnight. After cooling to room
35
36 temperature, the solution was extracted with diethyl ether twice, then placed chilled at 4°C for 4
37
38 hours. The solid was collected by filtration and dried to give 8-benzyloxyquinoline-5-sulfonic
39
40 acid **54** (6.3 g, 48% yield) a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.10 - 9.17 (m,
41
42 1 H) 8.84 (dd, *J* = 4.2, 1.6 Hz, 1 H) 7.88 (d, *J* = 8.1 Hz, 1 H) 7.52 - 7.60 (m, 3 H) 7.39 - 7.46 (m,
43
44 2 H) 7.32 - 7.39 (m, 1 H) 7.20 (d, *J* = 8.1 Hz, 1 H) 5.32 (s, 2 H). MS (ES+) *m/z* 316.0 [M+H]⁺
45
46
47
48
49

50 *Step 2.* To a stirring solution of 8-benzyloxyquinoline-5-sulfonic acid **54** (6.3 g, 20.0
51
52 mmol) and thionyl chloride (100 mL, 20.0 mmol) was added DMF (3 drops). The solution was
53
54 heated at 80°C for 3 hours. The solution was cooled to room temperature and concentrated.
55
56
57
58
59
60

1
2
3 Toluene (50 ml) was added and the resultant mixture concentrated to azeotrope excess thionyl
4 chloride, and the resultant suspension was concentrated to give 8-benzyloxyquinoline-5-sulfonyl
5 chloride **55** (8.9 g) as a pale yellow solid. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ ppm 5.76 (s, 2 H) 7.30
6 - 7.45 (m, 13 H) 7.62 - 7.70 (m, 6 H) 8.25 (dd, $J = 8.84, 5.05$ Hz, 3 H) 8.50 - 8.56 (m, 3 H) 9.59
7 (dd, $J = 5.05, 1.26$ Hz, 3 H) 9.66 (dd, $J = 8.84, 1.01$ Hz, 3 H). MS (ES+) m/z 334.9 $[\text{M}+\text{H}]^+$
8
9
10
11
12
13
14

15 *Step 3.* To a stirring solution of 8-benzyloxyquinoline-5-sulfonyl chloride **55** (14 g, 41.9
16 mmol) in THF (100 mL) was added triphenylphosphine (33 g, 125.8 mmol). An exotherm was
17 observed and reaction mixture turned dark brown. The solution was heated at 60°C for 60
18 minutes then cooled to room temperature. Diethyl ether (50 ml) was added, a precipitate formed
19 and solids collected by filtration. After dissolving the precipitate in DMF, sodium borohydride
20 (2.0 g, 54.5 mmol) was added. The solution was stirred at room temperature for 30 minutes.
21 Cyclopentyl iodide (8.2 g, 41.9 mmol) was added and the reaction heated to 60°C for two hours.
22 The solution was cooled to room temperature and diluted with water (50 mL). The solution was
23 extracted with ethyl acetate (3 X 50 mL). The combined organic washes were extracted with
24 water (3 X 40 mL), brine (1 X 50 mL), dried over sodium sulfate, filtered and concentrated. The
25 residue was purified automated normal-phase chromatography and eluted with ethyl
26 acetate/hexane (0-50%) to give 8-benzyloxy-5-cyclopentylsulfanyl-quinoline **56** (2.3 g, 16%
27 yield) as a colorless oil. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ ppm 8.99 - 9.05 (m, 1 H) 8.86 (dd, $J =$
28 8.5, 1.6 Hz, 1 H) 7.65 (d, $J = 8.1$ Hz, 1 H) 7.50 - 7.59 (m, 3 H) 7.29 - 7.43 (m, 3 H) 6.96 - 7.03
29 (m, 1 H) 5.44 - 5.50 (m, 2 H) 3.36 - 3.47 (m, 1 H) 1.73 - 1.94 (m, 4 H) 1.49 - 1.67 (m, 4 H). MS
30 (ES+) m/z 336.1 $[\text{M}+\text{H}]^+$.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53 *Step 4.* To a stirring solution of 8-benzyloxy-5-cyclopentylsulfanyl-quinoline **56** (5.3 g,
54 15.8 mmol) in DCM (150 mL), 3-chlorobenzenecarboxylic acid (1.1 g, 4.7 mmol) was
55
56
57
58
59
60

1
2
3 added. The solution was stirred at room temperature for 30 minutes. Sodium sulfite solution (1
4 M, 30 mL) was added. The solution was extracted with sodium hydroxide solution (1.0 N, 2 X
5 60 mL), brine (1 X 60 mL) and dried over sodium sulfate. The solution was filtered and
6
7 concentrated. The residue was purified by automated normal-phase chromatography and eluted
8
9 with methanol/dichloromethane (0-4%) to give 8-benzyloxy-5-cyclopentylsulfonyl-quinoline **57**
10 (3.7 g, 61% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.97 - 9.04 (m, 2 H)
11
12 8.22 (d, *J* = 8.3 Hz, 1 H) 7.75 - 7.81 (m, 1 H) 7.55 - 7.62 (m, 2 H) 7.36 - 7.53 (m, 4 H) 5.42 (s, 2
13
14 H) 3.78 - 3.90 (m, 1 H) 1.47 - 1.95 (m, 8 H). MS (ES+) *m/z* 368.1 [M+H]⁺.
15
16
17
18
19
20
21

22 *Step 5.* To a stirring solution of 8-benzyloxy-5-cyclopentylsulfonyl-quinoline **57** (3.7 g,
23 10.1 mmol) in acetic acid (50 mL), hydrobromic acid (34 mL, 201.9 mmol) was added. The
24 solution was stirred at 100°C for 5 hours. The solution was cooled to room temperature and
25 concentrated to half of its volume. The solution was washed with diethyl ether and etheral wash
26 chilled at 4°C overnight. The white precipitate was collected by filtration and dried to give the
27 desired product as a hydrobromide salt. The solid was dissolved in aqueous sodium hydroxide
28 solution (0.5 N, 50 mL). The solution was filtered to remove particulates and then filtrate
29 adjusted to slightly acidic by adding hydrochloric acid solution (1 N, ~pH 5-6). The solution
30 was triturated with saturated sodium bicarbonate aqueous solution to provide a white
31 precipitate. The precipitate was isolated by filtration, washed with water three times, and air-
32 dried to give 5-cyclopentylsulfonylquinolin-8-ol **11** (1.7 g, 61% yield) as a free base. ¹H NMR
33 (400 MHz, DMSO-*d*₆) δ ppm 11.17 (br. s., 1 H) 8.97 - 9.05 (m, 2 H) 8.13 (d, *J* = 8.3 Hz, 1 H)
34
35 7.75 - 7.84 (m, 1 H) 7.24 (d, *J* = 8.1 Hz, 1 H) 3.81 (quin, *J* = 7.6 Hz, 1 H) 1.45 - 1.94 (m, 8 H).
36
37 MS (ES+) *m/z* 278.0 [M+H]⁺.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **5-(Pyrrolidin-1-ylsulfonyl)quinoline-8-ol (12)**. *Step 1*. 8-Fluoroquinoline **31** (10.5 g,
4 71.3 mmol) was added dropwise with stirring to chlorosulfonic acid (10 mL, 150.4 mmol). The
5
6 resulting mixture was stirred at 110°C for ~30 hours. The crude mixture was added dropwise to
7
8 ice water with stirring, causing solids to precipitate. Solids were collected by filtration, rinsed
9
10 with a small amount of water and dried under reduced pressure to afford 8-fluoroquinoline-5-
11
12 sulfonyl chloride **32** (12.5 g, 71% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm
13
14 9.36 (d, *J* = 8.59 Hz, 1 H) 9.04 (d, *J* = 3.28 Hz, 1 H) 8.02 (dd, *J* = 8.08, 5.31 Hz, 1 H) 7.82 (dd,
15
16 *J* = 8.72, 4.42 Hz, 1 H) 7.62 (dd, *J* = 10.61, 8.08 Hz, 1 H). MS (ES+) *m/z* 246.0 [M+H]⁺.
17
18
19
20

21 *Step 2*. To a suspension of 8-fluoroquinoline-5-sulfonyl chloride **32** (2.0 g, 8.1 mmol) in
22
23 THF (25 mL) was added DIPEA (2.8 mL, 16.2 mmol) at ambient temperature. Pyrrolidine (0.67
24
25 mL, 8.1 mmol) was added slowly dropwise as a solution in THF (5 mL). Meanwhile to a slurry
26
27 of sodium hydride (977 mg, 24.4 mmol) in THF (15 mL) was added 2-(trimethylsilyl)ethanol
28
29 (3.5 mL, 24.4 mmol). After complete addition of pyrrolidine to the 8-fluoroquinoline-5-sulfonyl
30
31 chloride a HPLC chromatogram confirmed the first step was complete. This sulfonamide
32
33 mixture was added to the slurry of trimethylsilylethanol sodium salt slowly dropwise. The
34
35 reaction was allowed to stir at ambient temperature for one hour. The reaction was quenched by
36
37 addition of water. Ethyl acetate was added and the layers were separated. The aqueous layer
38
39 was extracted (3 X 50 mL) with ethyl acetate. The organics were combined, dried over sodium
40
41 sulfate, concentrated to a residue and purified by silica chromatography (100 g) eluting 0-100%
42
43 ethyl acetate/hexanes. Isolated 2.2 g (70% yield) of trimethyl-[2-[(5-pyrrolidin-1-ylsulfonyl-8-
44
45 quinolyl)oxy]ethyl]silane **58**. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.88 - 9.08 (m, 2 H) 8.14
46
47 (d, *J* = 8.59 Hz, 1 H) 7.75 (dd, *J* = 8.84, 4.04 Hz, 1 H) 7.35 (d, *J* = 8.59 Hz, 1 H) 4.21 - 4.47 (m,
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 2 H) 3.06 - 3.24 (m, 4 H) 1.59 - 1.79 (m, 4 H) 1.15 - 1.33 (m, 2 H) 0.13 (s, 9 H). MS (ES+) m/z
4
5 379.0 [M+H]⁺.
6

7
8 *Step 3.* To a solution of trimethyl-[2-[(5-pyrrolidin-1-ylsulfonyl-8-
9
10 quinolyl)oxy]ethyl]silane **58** (2.1 g, 5.6 mmol) dissolved in THF (30 mL) was added
11
12 tetrabutylammonium fluoride (5.6 mL, 5.6 mmol) as a 1M solution in THF. The mixture was
13
14 allowed to stir for 1 hour. Excess solvent was removed by rotary evaporator and resultant
15
16 residue partitioned between water and dichloromethane. Extraction of the aqueous material was
17
18 accomplished by (3 X 30 mL) dichloromethane. The organic layer was dried over sodium
19
20 sulfate and concentrated to an oil. The material was purified by silica chromatography eluting 0-
21
22 10% dichloromethane/methanol to provide 5-(pyrrolidin-1-ylsulfonyl)quinoline-8-ol **12**, 548 mg
23
24 (34% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 11.02 (br. s., 1 H) 9.04 (dd, $J = 8.72, 1.64$
25
26 Hz, 1 H) 8.98 (dd, $J = 3.79, 1.26$ Hz, 1 H) 8.09 (d, $J = 8.34$ Hz, 1 H) 7.78 (dd, $J = 8.84, 4.29$ Hz,
27
28 1 H) 7.20 (d, $J = 8.34$ Hz, 1 H) 3.07 - 3.23 (m, 4 H) 1.71 (dt, $J = 6.51, 3.44$ Hz, 4 H). MS (ES+)
29
30 m/z 279.0 [M+H]⁺.
31
32
33
34
35
36
37

38 **5-((3-(4-Fluorophenyl)pyrrolidin-1-yl)sulfonyl)quinoline-8-ol (13).** *Step 1.* To a
39
40 suspension of 8-fluoroquinoline-5-sulfonyl chloride **32** (150 mg, 0.61 mmol) in THF (4 mL) was
41
42 added DIPEA (319 μ L, 1.8 mmol) dropwise. After complete addition 3-(4-
43
44 fluorophenyl)pyrrolidine hydrochloride (123 mg, 0.61 mmol) was added in one portion. The
45
46 reaction was quenched by addition of water and partitioned between water and
47
48 dichloromethane. The aqueous was extracted with dichloromethane (2 X 3 mL), organics
49
50 combined, dried over sodium sulfate and concentrated to a residue. Purification was
51
52 accomplished by automated silica chromatography eluting with ethyl acetate/heptane at a 0-80%
53
54
55
56
57
58
59
60

1
2
3 gradient over 20 minutes. 8-Fluoro-5-[3-(4-fluorophenyl)pyrrolidin-1-yl]sulfonyl-quinoline **59**
4
5 was isolated (210 mg, 92% yield). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 9.08 - 9.16 (m, 2 H)
6
7 8.26 (dd, $J = 8.34, 4.80$ Hz, 1 H) 7.83 - 7.90 (m, 1 H) 7.78 (dd, $J = 9.98, 8.46$ Hz, 1 H) 7.18 -
8
9 7.27 (m, 2 H) 7.00 - 7.11 (m, 2 H) 3.75 (dd, $J = 9.35, 7.58$ Hz, 1 H) 3.52 (td, $J = 8.97, 3.03$ Hz, 1
10
11 H) 3.27 - 3.42 (m, 2 H) 3.09 - 3.20 (m, 1 H) 2.14 - 2.28 (m, 1 H) 1.83 - 2.02 (m, 1 H). MS (ES+)
12
13 m/z 375.0 $[\text{M}+\text{H}]^+$.
14
15

16
17 *Step 2.* A suspension of sodium hydride (67 mg, 1.6 mmol) in THF (2 ml) was added 2-
18
19 (trimethylsilyl)ethanol (0.24 mL, 1.6 mmol) slowly, dropwise over 5 minutes. The resultant
20
21 suspension was added to a solution of 8-fluoro-5-[3-(4-fluorophenyl)pyrrolidin-1-yl]sulfonyl-
22
23 quinoline **59** (210 mg, 0.56 mmol) in THF (3 mL) and resultant mixture was allowed to stir at
24
25 ambient temperature for 30 minutes. The reaction was quenched by addition of water and
26
27 partitioned between water and dichloromethane. The aqueous was extracted with
28
29 dichloromethane (2 X 3 mL), organics combined, dried over sodium sulfate and concentrated to
30
31 a residue. Purification was accomplished by automated silica chromatography eluting with ethyl
32
33 acetate/heptane at a 0-60% gradient over 12 minutes to provide 2-[[5-[3-(4-
34
35 fluorophenyl)pyrrolidin-1-yl]sulfonyl-8-quinolyl]oxy]ethyl-trimethyl-silane **60** (212 mg, 80%
36
37 yield). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 9.02 (dd, $J = 8.84, 1.77$ Hz, 1 H) 8.95 - 8.99 (m,
38
39 1 H) 8.17 (d, $J = 8.59$ Hz, 1 H) 7.74 (dd, $J = 8.72, 4.17$ Hz, 1 H) 7.34 (d, $J = 8.59$ Hz, 1 H) 7.19
40
41 (dd, $J = 8.72, 5.68$ Hz, 2 H) 7.00 - 7.10 (m, 2 H) 4.39 (d, $J = 7.58$ Hz, 2 H) 3.65 - 3.74 (m, 1 H)
42
43 3.41 - 3.52 (m, 1 H) 3.31 (d, $J = 8.84$ Hz, 2 H) 3.08 (s, 1 H) 2.14 - 2.26 (m, 1 H) 1.80 - 1.95 (m,
44
45 1 H) 1.27 (dd, $J = 8.72, 7.71$ Hz, 2 H) 0.10 - 0.17 (m, 9 H). MS (ES+) m/z 473.0 $[\text{M}+\text{H}]^+$.
46
47
48
49
50

51
52 *Step 3.* To a solution of 2-[[5-[3-(4-fluorophenyl)pyrrolidin-1-yl]sulfonyl-8-
53
54 quinolyl]oxy]ethyl-trimethyl-silane **60** (210 mg, 0.44 mmol) in DMF (2 mL) was added cesium
55
56
57
58
59
60

1
2
3 fluoride (202 mg, 1.3 mmol). The vial was heated to 60°C for 2 hours then warmed to 75°C for 2
4 additional hours. The mixture was partitioned between brine and ethyl acetate. The layers were
5 separated and aqueous extracted with ethyl acetate (1 X 5 mL), organics combined, dried over
6 sodium sulfate and concentrated to a DMF suspended residue. To this residue was added diethyl
7 ether and resultant suspension filtered. The filtrate was concentrated, suspended in heptane and
8 collected by filtration to yield 5-((3-(4-fluorophenyl)pyrrolidin-1-yl)sulfonyl)quinoline-8-ol **13**,
9 111 mg (67% yield) as a free base. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.97 - 11.14 (m, 1
10 H) 9.05 (dd, *J* = 8.72, 1.64 Hz, 1 H) 8.98 (dd, *J* = 4.04, 1.52 Hz, 1 H) 8.13 (d, *J* = 8.34 Hz, 1 H)
11 7.77 (dd, *J* = 8.84, 4.04 Hz, 1 H) 7.20 (dt, *J* = 8.53, 2.81 Hz, 3 H) 7.00 - 7.11 (m, 2 H) 3.68 (dd,
12 *J* = 9.47, 7.45 Hz, 1 H) 3.40 - 3.50 (m, 1 H) 3.24 - 3.33 (m, 2 H) 3.02 - 3.12 (m, 1 H) 2.17 (d, *J*
13 = 3.28 Hz, 1 H) 1.85 (dd, *J* = 12.51, 9.73 Hz, 1 H). MS (ES+) *m/z* 373.0 [M+H]⁺.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **8-Methoxy-5-(pyrrolidin-1-ylsulfonyl)quinoline (14)**. 8-fluoroquinoline-5-sulfonyl
32 chloride **32** (50 mg, 0.20 mmol) was dissolved in DCM (1 mL). To this solution was added a
33 solution of pyrrolidine (17 μL, 0.20 mmol) and DIPEA (71 μL, 0.41 mmol) in DCM (0.5 mL).
34 The resulting mixture was stirred at room temperature for 5 min. Added sodium methoxide, 25
35 wt % in MeOH (200 μL, 3.5 mmol) and stirred at room temperature for 2 h. The reaction
36 mixture was purified directly by automated normal-phase chromatography (20-100%
37 EtOAc/hexanes (7% MeOH additive), 10 g silica gel cartridge). The product-containing fractions
38 were combined and the solvent removed in vacuo to give 8-methoxy-5-pyrrolidin-1-ylsulfonyl-
39 quinoline **14** (47 mg, 79% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.02
40 (dd, *J* = 8.72, 1.64 Hz, 1 H) 8.97 (dd, *J* = 4.17, 1.64 Hz, 1 H) 8.17 (d, *J* = 8.34 Hz, 1 H) 7.76
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(dd, $J = 8.84, 4.04$ Hz, 1 H) 7.34 (d, $J = 8.59$ Hz, 1 H) 4.07 (s, 3 H) 3.15 - 3.21 (m, 4 H) 1.69 - 1.75 (m, 4 H). MS (ES+) m/z 293.0 $[M+H]^+$.

5-(Ethylsulfonyl)quinoline-8-ol (15). *Step 1.* To a stirring solution of 8-benzyloxyquinoline-5-sulfonyl chloride **55** (1.5 g, 4.4 mmol) in THF (50 mL), triphenylphosphine (3.5 g, 13.4 mmol) was added. The solution was heated at 60°C for 60 minutes. The solution was cooled to room temperature and diethyl ether (50 mL) added. The precipitate was collected by filtration. The precipitate was dissolved in DMF (20 mL) and to this solution was added sodium borohydride (220 mg, 5.8 mmol). The solution was stirred at room temperature for 30 minutes. Iodoethane (1.0 g, 6.7 mmol) was added and resultant mixture stirred at 60°C for two hours. The solution was cooled to room temperature and water (50 mL) added. The solution was extracted with ethyl acetate (3 X 50 mL). The combined organic solution was extracted with water (3 X 40 mL), brine (1 X 50 mL) and dried over sodium sulfate. The organic solution was filtered and concentrated. The residue was purified by automated normal-phase chromatography and eluted with ethyl acetate/hexane (0-80%) to give 8-benzyloxy-5-ethylsulfanyl-quinoline **61** (580 mg, 43% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 8.99 - 9.05 (m, 1 H), 8.82 (dd, $J = 8.6, 1.8$ Hz, 1 H), 7.59 - 7.66 (m, 1 H), 7.49 - 7.58 (m, 3 H), 7.29 - 7.44 (m, 3 H), 6.96 - 7.04 (m, 1 H), 5.43 - 5.50 (m, 2 H), 2.77 - 2.90 (m, 2 H), 1.16 - 1.27 (m, 3 H). MS (ES+) m/z 296.1 $[M+H]^+$.

Step 2. To a stirring solution of 8-benzyloxy-5-ethylsulfanyl-quinoline **61** (400 mg, 1.3 mmol) in DCM (50 mL), 3-chlorobenzenecarboperoxoic acid (0.84 g, 3.4 mmol) was added. The solution was stirred at room temperature for 30 minutes. Sodium sulfite solution (1 M, 30 mL) was added. The solution was diluted with DCM (30 mL) and extracted with sodium hydroxide

1
2
3 solution (1 N, 2 X 30 mL), brine (1 X 30 mL) and dried over sodium sulfate. The solution was
4 filtered and concentrated. The residue was purified by automated normal-phase chromatography
5 and eluted with methanol/DCM (0-8%) to give a white solid. Mixed fractions were pooled and
6 purified by automated reversed-phase chromatography and eluted with 5-95% acetonitrile/water,
7 with 0.05% TFA as modifier to give white solid which was combined with clean, pooled
8 fractions from the previous purification to provide 8-benzyloxy-5-ethylsulfonyl-quinoline **62**
9 (206 mg, 44% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.92 - 9.04 (m, 2
10 H), 8.21 (d, *J* = 8.6 Hz, 1 H), 7.80 (dd, *J* = 8.7, 4.2 Hz, 1 H), 7.35 - 7.63 (m, 6 H), 5.43 (s, 2 H),
11 3.39 (q, *J* = 7.4 Hz, 11 H), 1.09 (t, *J* = 7.3 Hz, 3 H). MS (ES+) *m/z* 328.1 [M+H]⁺.
12
13
14
15
16
17
18
19
20
21
22
23

24 *Step 3.* To a stirring solution of 8-benzyloxy-5-ethylsulfonyl-quinoline **62** (200 mg, 0.61
25 mmol) in acetic acid (1 mL), hydrobromic acid (2.0 mL, 12.2 mmol) was added. The solution
26 was stirred at 100°C for 16 hrs. The solution was cooled to room temperature and diluted with
27 diethyl ether (2 mL). A precipitate formed and was collected by filtration to give 5-
28 ethylsulfonylquinolin-8-ol hydrobromide **15** (132 mg, 67% yield) as a white solid. ¹H NMR
29 (400 MHz, DMSO-*d*₆) δ ppm 9.14 (d, *J* = 8.6 Hz, 1 H), 9.02 - 9.08 (m, 1 H), 8.19 (d, *J* = 8.3 Hz,
30 1 H), 7.92 (dd, *J* = 8.7, 4.4 Hz, 1 H), 7.32 (d, *J* = 8.3 Hz, 1 H), 3.38 (q, *J* = 7.3 Hz, 2 H), 1.09 (t,
31 *J* = 7.3 Hz, 3 H). MS (ES+) *m/z* 238.1 [M+H]⁺.
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **5-((2-Benzylpyrrolidin-1-yl)sulfonyl)quinoline-8-ol (16).** *Step 1.* 8-fluoroquinoline-5-
46 sulfonyl chloride **32** (50 mg, 0.20 mmol) was suspended in THF (1 mL). To this suspension was
47 added a solution of DIPEA (71 μL, 0.41 mmol) and 2-benzylpyrrolidine (33 mg, 0.20 mmol) in
48 THF (0.5 mL). The resulting mixture was stirred at room temperature for 15 min. To this
49 mixture was added a suspension of 2-(trimethylsilyl)ethanol (29 μL, 0.20 mmol) and sodium
50
51
52
53
54
55
56
57
58
59
60

1
2
3 hydride (8 mg, 0.20 mmol) in THF (1 mL) and stirred at ambient temperature for 1 hour. A
4
5 second suspension of 2-(trimethylsilyl)ethanol (29 μ L, 0.204 mmol) and sodium hydride (8 mg,
6
7 0.20 mmol) in THF (1 mL) was added and stirred at ambient temperature for an additional hour.
8
9 Treated reaction mixture with saturated NaHCO₃ and CHCl₃, agitated vigorously and poured into
10
11 a phase separator tube. The organic layer was concentrated in vacuo and the residue purified by
12
13 automated normal-phase chromatography (20-100% EtOAc/hexanes, 4 g silica gel cartridge).
14
15 The product-containing fractions were combined, and the solvent removed in vacuo to provide 2-
16
17 [[5-(2-benzylpyrrolidin-1-yl)sulfonyl-8-quinolyl]oxy]ethyl-trimethyl-silane **63** (69 mg, 72%
18
19 yield) as a colorless oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.06 (dd, *J* = 8.72, 1.64 Hz, 1
20
21 H) 8.97 (dd, *J* = 4.17, 1.64 Hz, 1 H) 8.23 (d, *J* = 8.59 Hz, 1 H) 7.76 (dd, *J* = 8.84, 4.04 Hz, 1 H)
22
23 7.36 (d, *J* = 8.59 Hz, 1 H) 7.22 - 7.28 (m, 2 H) 7.12 - 7.21 (m, 3 H) 4.34 - 4.42 (m, 2 H) 3.84 -
24
25 3.92 (m, 1 H) 3.18 - 3.32 (m, 2 H) 2.88 (dd, *J* = 13.14, 3.79 Hz, 1 H) 2.62 (dd, *J* = 13.01, 9.73
26
27 Hz, 1 H) 1.65 - 1.77 (m, 1 H) 1.41 - 1.60 (m, 3 H) 1.23 - 1.30 (m, 2 H) 0.08 - 0.14 (m, 9 H). MS
28
29 (ES+) *m/z* 469.2 [M+H]⁺.
30
31
32
33
34
35

36 *Step 2.* To a solution of 2-[[5-(2-benzylpyrrolidin-1-yl)sulfonyl-8-quinolyl]oxy]ethyl-
37
38 trimethyl-silane **63** (46 mg, 0.097 mmol) in DMF (0.5 mL) was added cesium fluoride (44 mg,
39
40 0.29 mmol). The contents were stirred at 60°C for 4 h, then at ambient temperature for 48 h (for
41
42 convenience). Added water and extracted with EtOAc (2 X). The organic layers were combined,
43
44 washed with brine, dried with MgSO₄, filtered and the solvent removed in vacuo to give 5-(2-
45
46 benzylpyrrolidin-1-yl)sulfonylquinolin-8-ol **16** (30 mg, 84% yield) as a tan solid. ¹H NMR (400
47
48 MHz, DMSO-*d*₆) δ ppm 11.05 (br. s., 1 H) 9.09 (dd, *J* = 8.84, 1.52 Hz, 1 H) 8.98 (dd, *J* = 4.29,
49
50 1.52 Hz, 1 H) 8.18 (d, *J* = 8.34 Hz, 1 H) 7.79 (dd, *J* = 8.84, 4.04 Hz, 1 H) 7.10 - 7.31 (m, 6 H)
51
52
53
54
55
56
57
58
59
60

1
2
3 3.86 (t, $J = 8.46$ Hz, 1 H) 3.15 - 3.30 (m, 3 H) 2.84 - 2.95 (m, 1 H) 2.62 (dd, $J = 13.14, 9.60$ Hz,
4 1 H) 1.63 - 1.76 (m, 1 H) 1.36 - 1.60 (m, 3 H). MS (ES+) m/z 369.0 $[M+H]^+$.
5
6
7
8
9
10

11 **5-((4-(Trifluoromethyl)phenyl)sulfonyl)quinolin-8-ol (17)**. *Step 1*. A mixture of 8-
12 benzyloxy-5-bromo-quinoline **51** (compound 8, step 1) (5.0 g, 15.9 mmol), 2-
13 trimethylsilylethanethiol (3.8 mL, 23.8 mmol), tris(dba)dipalladium (729 mg, 0.80 mmol),
14 Xantphos (460.4 mg, 0.80 mmol) and cesium carbonate (15.5 g, 47.7 mmol) in dioxane (125
15 mL) was degassed with nitrogen. The resultant mixture was heated at 100°C overnight. The
16 mixture was passed through a plug of silica eluting with ethyl acetate and concentrated to a
17 residue. The residue was purified by automated normal-phase chromatography eluting 0-30%
18 ethyl acetate/hexanes to yield 5.8 g (99%) of 2-[(8-benzyloxy-5-quinolyl)sulfanyl]ethyl-
19 trimethyl-silane **64** as a golden oil. ^1H NMR (400 MHz, DMSO- d_6) δ ppm 8.88 - 8.93 (m, 1 H)
20 8.64 - 8.71 (m, 1 H) 7.62 - 7.72 (m, 2 H) 7.52 - 7.59 (m, 2 H) 7.40 - 7.48 (m, 2 H) 7.38 (d, $J =$
21 6.06 Hz, 1 H) 7.27 - 7.33 (m, 1 H) 5.33 (br. s., 2 H) 2.86 - 2.96 (m, 2 H) 0.75 - 0.84 (m, 2 H) -
22 0.08 - 0.00 (m, 9 H). MS (ES+) m/z 368.2 $[M+H]^+$.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 *Step 2*. A mixture of 2-[(8-benzyloxy-5-quinolyl)sulfanyl]ethyl-trimethyl-silane **64** (5.2
40 g, 11.3 mmol), cesium carbonate (11.1 g, 34.1 mmol), Xantphos (329 mg, 0.57 mmol) and
41 tris(dba)dipalladium (521 mg, 0.57 mmol) was suspended in dioxane (100 mL). To this mixture
42 was added 1-bromo-4-(trifluoromethyl)benzene (1.9 mL, 13.6 mmol) followed by
43 tetrabutylammonium fluoride (1 M solution in THF) (13.6 mL, 13.6 mmol). The resultant
44 mixture was degassed (X 2) with nitrogen. With a nitrogen vent afixed, the reaction mixture was
45 heated to 75°C for 2 hours. The reaction was cooled to ambient temperature and filtered through
46 a plug of silica gel. The filtrate was concentrated and purified using silica gel chromatography
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 eluting 0-30% ethyl acetate/hexanes to provide 8-benzyloxy-5-[4-
4
5 (trifluoromethyl)phenyl]sulfonyl-quinoline **65**, 5.1 g (assume quantitative yield). The material
6
7 was taken on in subsequent reactions without additional purification and or characterization. ¹H
8
9 NMR (400 MHz, CDCl₃) δ ppm 9.04 (dd, J=4.17, 1.64 Hz, 1 H) 8.57 (dd, J=8.59, 1.77 Hz, 1 H)
10
11 7.83 (d, J=8.08 Hz, 1 H) 7.54 - 7.60 (m, 2 H) 7.50 (dd, J=8.59, 4.04 Hz, 1 H) 7.32 - 7.46 (m, 5
12
13 H) 7.11 (d, J=8.08 Hz, 1 H) 7.02 (d, J=8.08 Hz, 2 H) 5.52 (s, 2 H). MS (ES+) *m/z* 412.0
14
15 [M+H]⁺.
16
17
18
19

20 *Step 3.* To a stirred solution of 8-benzyloxy-5-[4-(trifluoromethyl)phenyl]sulfonyl-
21
22 quinoline **65** (5.1 g, 12.5 mmol) in DCM (100 mL), 3-chlorobenzenecarboperoxoic acid (4.5 g,
23
24 26.2 mmol) was added. The solution was stirred at room temperature for 3 hours. Additional
25
26 mCPBA (1 g) was added and the resultant reaction allowed to stir at ambient temperature
27
28 overnight. Sodium bisulfite (10%, 50 mL) was added and stirred for one hour. The layers were
29
30 separated. The organic solution was stirred with 10% (2.5 M) NaOH for 1 hour and subsequent
31
32 layers separated. The organic solution was dried over sodium sulfate and concentrated to a
33
34 residue. The residue was purified by silica chromatography eluting 0-50% ethyl
35
36 acetate/hexanes. Isolated a yellow foam. The foam was dissolved in diethyl ether (20
37
38 mL). Solids formed and the suspension heated to reflux. The suspension was allowed to cool to
39
40 ambient temperature and solids collected by filtration, washed with ether and hexanes to yield 8-
41
42 benzyloxy-5-[4-(trifluoromethyl)phenyl]sulfonyl-quinoline **66**, 3.7 g (68% yield). ¹H NMR (400
43
44 MHz, DMSO-*d*₆) δ ppm 8.96 (dd, *J* = 4.17, 1.64 Hz, 1 H) 8.85 (dd, *J* = 8.72, 1.64 Hz, 1 H) 8.57
45
46 (d, *J* = 8.59 Hz, 1 H) 8.20 (d, *J* = 8.34 Hz, 2 H) 7.97 (d, *J* = 8.34 Hz, 2 H) 7.72 (dd, *J* = 8.84,
47
48 4.04 Hz, 1 H) 7.53 - 7.62 (m, 3 H) 7.36 - 7.49 (m, 3 H) 5.44 (s, 2 H). MS (ES+) *m/z* 444.0
49
50 [M+H]⁺.
51
52
53
54
55
56
57
58
59
60

1
2
3 *Step 4.* A solution of 8-benzyloxy-5-[4-(trifluoromethyl)phenyl]sulfonyl-quinoline **66**
4 (3.7 g, 8.5 mmol) and 6 M aqueous hydrochloric acid (30 mL, 180 mmol) was heated to 100°C
5 for 1.5 hours. Dioxane (40 mL) was added to aid solubility. The mixture was heated to 100°C
6 overnight. The reaction was cooled to ambient temperature and pH adjusted to ~5 with 10%
7 NaOH aqueous solution. The slurry was filtered and solids washed with water to yield 5-((4-
8 (Trifluoromethyl)phenyl)sulfonyl)quinolin-8-ol **17**, 2.9 g (97% yield). ¹H NMR (400 MHz,
9 DMSO-*d*₆) δ ppm 11.35 - 11.51 (m, 1 H) 8.96 (dd, *J* = 4.30, 1.52 Hz, 1 H) 8.86 (dd, *J* = 8.84,
10 1.52 Hz, 1 H) 8.47 (d, *J* = 8.34 Hz, 1 H) 8.13 - 8.22 (m, 2 H) 7.96 (d, *J* = 8.84 Hz, 2 H) 7.74 (dd,
11 *J* = 8.84, 4.04 Hz, 1 H) 7.30 (d, *J* = 8.59 Hz, 1 H). MS (ES+) *m/z* 354.0 [M+H]⁺.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

27 **7-Fluoro-5-((4-(trifluoromethyl)phenyl)sulfonyl)quinolin-8-ol (18).** *Step 1.* A
28 mixture of 2-amino-6-fluoro-phenol (1.8 mL, 13.9 mmol), glycerol (2.5 mL, 34.7 mmol)
29 and sodium 3-nitrobenzenesulfonate (3.7 g, 16.7 mmol) was treated with 70% sulfuric acid
30 (11.9 mL, 224.5 mmol) and heated to reflux (140°C) for 1 hour. The mixture was cooled,
31 diluted with water (75 mL) and basified with 50% sodium hydroxide aqueous solution,
32 pH~8. Filtration through Celite gave a solid which was washed with dichloromethane. The
33 aqueous filtrate was washed with dichloromethane and combined extracts evaporated to give an
34 oily residue. The oily residue and brown solids were combined and purified by silica
35 chromatography (50 g) eluting 0-20% dichloromethane/methanol to yield 7-fluoroquinolin-8-ol
36 **41** as a pale yellow solid, 1.3 g, 56%. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.42 - 7.49 (m, 1
37 H) 7.50 - 7.57 (m, 2 H) 8.37 (dd, *J*=8.34, 1.77 Hz, 1 H) 8.90 (dd, *J*=4.30, 1.52 Hz, 1 H) 10.26 (br.
38 s., 1 H). MS (ES+) *m/z* 164.2 [M+H]⁺.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 *Step 2.* A solution of 7-fluoroquinolin-8-ol **41** (4.0 g, 24.5 mmol) and N-iodosuccinimide
4 (6.6 g, 29.4 mmol) in chloroform was vigorously stirred at 40°C. After 30 minutes the reaction
5 was diluted with dichloromethane, extracted 10% sodium thiosulfate solution (2 X 20 mL) and
6 dried over sodium sulfate. The 7-fluoro-5-iodo-quinolin-8-ol **67**, isolated as pale yellow solids
7 were taken on in subsequent reactions without additional purification. (6.5 g, 92% yield). ¹H
8 NMR (400 MHz, CDCl₃) δ ppm 8.80 (dd, *J* = 4.17, 1.39 Hz, 1 H) 8.32 (dd, *J* = 8.59, 1.52 Hz, 1
9 H) 7.97 (d, *J* = 9.85 Hz, 1 H) 7.53 (dd, *J* = 8.59, 4.29 Hz, 1 H). MS (ES+) *m/z* 290.0 [M+H]⁺.

10
11
12
13
14
15
16
17
18
19
20 *Step 3.* To a solution of sodium hydride (654 mg, 27.2 mmol) in DMF (100 mL) was
21 added 7-fluoro-5-iodo-quinolin-8-ol **67** (6.5 g, 22.7 mmol) as a solution in DMF. After 60
22 minutes benzyl bromide (3.2 mL, 27.2 mmol) was added slowly dropwise. The reaction was
23 maintained at ambient temperature. The reaction was allowed to stir overnight at ambient
24 temperature. The reaction was quenched by addition of water. Ethyl acetate was added and the
25 layers separated. The aqueous layer was extracted with ethyl acetate (2 X 20 mL), the organics
26 were combined and dried over sodium sulfate. Concentration gave an oily residue that was
27 purified by automated normal-phase chromatography (40 g) eluting 0-20% ethyl acetate/hexanes.
28 Isolated 8-benzyloxy-7-fluoro-5-iodo-quinoline **42** as an orange oil that solidified upon standing
29 (6.3 g, 73% yield). ¹H NMR (400 MHz, CDCl₃) δ ppm 8.96 (dd, *J* = 4.04, 1.52 Hz, 1 H) 8.33
30 (dd, *J* = 8.46, 1.64 Hz, 1 H) 7.93 (d, *J* = 10.11 Hz, 1 H) 7.52 - 7.56 (m, 2 H) 7.49 (dd, *J* = 8.59,
31 4.04 Hz, 1 H) 7.31 - 7.38 (m, 3 H) 5.54 (s, 2 H). MS (ES+) *m/z* 380.0 [M+H]⁺.

32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48 *Step 4.* A mixture of 8-benzyloxy-7-fluoro-5-iodo-quinoline **42** (3 g, 7.9 mmol), 4-
49 (trifluoromethyl)thiophenol (1.6 mL, 11.8 mmol), tris(dba)dipalladium (362 mg, 0.40 mmol),
50 Xantphos (228 mg, 0.40 mmol) and cesium carbonate (7.7 g, 23.7 mmol) in dioxane (50 mL)
51 was degassed with nitrogen. The resultant mixture was heated at 90°C overnight. The mixture
52
53
54
55
56
57
58
59
60

1
2
3 was passed through a plug of silica eluting with ethyl acetate and concentrated to a residue. The
4
5 residue was purified automated normal-phase chromatography eluting 0-30% ethyl
6
7 acetate/hexanes to provide 2.0 g (60% yield) of 8-benzyloxy-7-fluoro-5-[4-
8
9 (trifluoromethyl)phenyl]sulfanyl-quinoline **68** which was taken in in the next step. MS (ES+)
10
11 m/z 430.0 $[M+H]^+$.
12
13
14

15 *Step 5.* To a mixture of 8-benzyloxy-7-fluoro-5-[4-(trifluoromethyl)phenyl]sulfanyl-
16
17 quinoline **68** (2.0 g, 4.8 mmol) in DCM (35 mL) at 0°C was added 3-
18
19 chlorobenzenecarboperoxoic acid (1.6 g, 9.5 mmol). The reaction was stirred at 0°C for 90
20
21 minutes. Additional mCPBA was added to drive the reaction to completion. Sodium sulfite (1.0
22
23 N solution, 50 mL) was added and stirred for 60 minutes then removed followed by sodium
24
25 hydroxide solution (1.0 N, 5 mL). The solution was stirred at room temperature overnight. The
26
27 aqueous phase was removed and organic solution was dried over sodium sulfate. The residue
28
29 was purified by normal phase chromatography eluting 0-50% ethyl acetate/hexanes to provide
30
31 1.3 g (60% yield) of 8-benzyloxy-7-fluoro-5-[4-(trifluoromethyl)phenyl]sulfonyl-quinoline **69** as
32
33 a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 9.04 (dd, $J = 4.04, 1.52$ Hz, 1 H) 8.94 (dd, J
34
35 = 8.84, 1.52 Hz, 1 H) 8.40 (d, $J = 11.12$ Hz, 1 H) 8.07 (d, $J = 8.34$ Hz, 2 H) 7.78 (d, $J = 8.84$
36
37 Hz, 2 H) 7.47 - 7.59 (m, 3 H) 7.29 - 7.41 (m, 3 H) 5.72 (s, 2 H). MS (ES+) m/z 462.0 $[M+H]^+$.
38
39
40
41
42

43 *Step 6.* A solution of 8-benzyloxy-7-fluoro-5-[4-(trifluoromethyl)phenyl]sulfonyl-
44
45 quinoline **69** (1.3 g, 2.9 mmol) and 6 M aqueous hydrochloric acid (20 mL, 120mmol) in dioxane
46
47 (20 mL) to aid solubility, was heated to 90°C for 3 hours. The solution was cooled to ambient
48
49 temperature and neutralized with 10% sodium hydroxide solution. Solids formed and were
50
51 collected by filtration. 7-Fluoro-5-((4-(trifluoromethyl)phenyl)sulfonyl)quinolin-8-ol **18** was
52
53 collected as tan solids (769 mg, 71% yield). ¹H NMR (400 MHz, CDCl₃) δ ppm 9.01 (dd, $J =$
54
55
56
57
58
59
60

1
2
3 8.84, 1.52 Hz, 1 H) 8.89 (dd, $J = 4.29, 1.52$ Hz, 1 H) 8.48 (d, $J = 10.36$ Hz, 1 H) 8.09 (d, $J =$
4
5 8.34 Hz, 2 H) 7.78 (d, $J = 8.34$ Hz, 2 H) 7.61 (dd, $J = 8.84, 4.29$ Hz, 1 H). MS (ES+) m/z 372.0
6
7 [M+H]⁺.
8
9

10
11
12
13 **7-Chloro-5-((4-fluorophenyl)sulfonyl)quinolin-8-ol (19).** *Step 1.* To a solution of 5-
14 bromoquinolin-8-ol **36** (6.9 g, 31.0 mmol) in chloroform (200 mL), N-chlorosuccinimide (4.5 g,
15 34.1 mmol) was added. The solution was stirred at 50°C for 20 hours. The solution was
16 concentrated and methanol (150 mL) was added. The solution was stirred for 10 minutes. The
17 precipitate was collected by filtration and dried to give 5-bromo-7-chloro-quinolin-8-ol **70** (6.3 g
18 78% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 11.08 (br. s., 2 H), 8.99 (br. s., 2 H), 8.46
19 (br. s., 1 H), 7.99 (br. s., 2 H), 7.77 (br. s., 1 H). MS (ES+) m/z 259.9 [M+H]⁺.
20
21
22
23
24
25
26
27
28
29
30

31 *Step 2.* To a solution of 5-bromo-7-chloro-quinolin-8-ol **70** (6.3 g, 24.3 mmol) and
32 benzyl bromide (3.1 mL, 26.8 mmol) in acetonitrile (100 mL) was added potassium carbonate
33 (5.0 g, 36.5 mmol). The solution was stirred at 50°C for 7 hours then cooled to room
34 (5.0 g, 36.5 mmol). The solution was stirred at 50°C for 7 hours then cooled to room
35 temperature. The solution was filtered. Water (50 ml) was added to the filtrate and an off-white
36 precipitate (crystals) formed. The solids were collected by filtration, washed with water and air-
37 dried to give 8-benzyloxy-5-bromo-7-chloro-quinoline **71** (7.6 g, 89% yield) as an off-white
38 solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 8.99 - 9.05 (m, 1 H), 8.51 (dd, $J = 8.6, 1.5$ Hz, 1 H),
39 7.84 - 7.88 (m, 1 H), 7.59 - 7.65 (m, 2 H), 7.52 - 7.59 (m, 1 H), 7.31 - 7.44 (m, 3 H), 5.50 (s, 2
40 H). MS (ES+) m/z 349.9 [M+H]⁺.
41
42
43
44
45
46
47
48
49
50
51

52 *Step 3.* 2-(Trimethylsilyl)ethane-1-thiol (1.7 g, 12.9 mmol) was added to a solution
53 mixture of 8-(benzyloxy)-5-bromo-7-chloro-quinoline **71** (3.0 g, 8.61 mmol),
54
55
56
57
58
59
60

1
2
3 tris(dba)dipalladium(0) (394 mg, 0.43 mmol), Xantphos (249 mg, 0.43 mmol) in 1,4-dioxane (58
4 mL) at room temperature. Cesium carbonate (8.4 g, 25.8 mmol) was added. The reaction was
5
6 sealed with a septum and heated to 90°C under nitrogen. Upon completion, the crude material
7
8 was poured into brine, extracted with ethyl acetate, dried over magnesium sulfate, filtered,
9
10 concentrated under reduced pressure. The residue was dissolved in a minimal quantity of
11
12 dichloromethane and purified by automated normal phase chromatography (0-30% ethyl
13
14 acetate/heptane). The product-containing fractions were combined, to give 8-(benzyloxy)-7-
15
16 chloro-5-((2-(trimethylsilyl)ethyl)thio)quinoline **72** (3.2 g, 93% yield). ¹H NMR (400 MHz,
17
18 CDCl₃) δ ppm 8.99 - 9.04 (m, 1H) 8.66 - 8.73 (m, 1H) 7.61 - 7.69 (m, 2H) 7.54 - 7.59 (m, 1H)
19
20 7.47 - 7.52 (m, 1H) 7.33 - 7.44 (m, 3H) 5.45 - 5.52 (m, 2H) 2.94 - 3.04 (m, 2H) 0.91 - 1.01 (m,
21
22 2H) 0.03 - 0.09 (m, 9H). MS (ES+) *m/z* 402.0 [M+H]⁺.
23
24
25
26
27
28

29 *Step 4.* 1,4-Dioxane was degassed by nitrogen displacement for 10 minutes prior to
30
31 initiating reaction. A solution of 8-(benzyloxy)-7-chloro-5-((2-
32
33 (trimethylsilyl)ethyl)thio)quinoline **72** (300 mg, 0.75 mmol) in 1,4-dioxane (5 mL) was added to
34
35 a 50 mL round bottom flask charged with a magnetic stir bar, 4-fluoroiodobenzene (199 mg, 0.90
36
37 mmol), tris(dba)dipalladium(0) (34 mg, 0.04 mmol), Xantphos (21 mg, 0.04 mmol). To this
38
39 solution mixture was added cesium carbonate (729 mg, 2.24 mmol) followed by
40
41 tetrabutylammonium fluoride (895 μL, 1M THF, 0.89 mmol). The flask was sealed with a
42
43 rubber septum, and heated to 90°C. Upon completion the reaction was cooled room temperature,
44
45 poured into brine, extracted with ethyl acetate, dried over sodium sulfate, filtered, and
46
47 concentrated under reduced pressure. The crude residue was dissolved in minimal
48
49 dichloromethane and purified by automated normal phase chromatography eluting with 0-35%
50
51 ethyl acetate/heptane over a 35 minute gradient. The product attained was 8-(benzyloxy)-7-
52
53
54
55
56
57
58
59
60

1
2
3 chloro-5-((4-fluorophenyl)thio)quinoline **73** (275 mg, 93% yield). ^1H NMR (400 MHz, CDCl_3)
4 δ ppm 8.99 - 9.04 (m, 1 H) 8.61 - 8.67 (m, 1 H) 7.59 - 7.68 (m, 4 H) 7.32 - 7.52 (m, 7 H) 7.19 -
5
6 7.27 (m, 2 H) 6.97 - 7.07 (m, 2 H) 5.50 - 5.58 (m, 2 H). MS (ES+) m/z 396.0 $[\text{M}+\text{H}]^+$.
7
8
9

10 *Step 5.* 3-chlorobenzoperoxoic acid (327 mg, 1.46 mmol) was added to a solution of 8-
11 (benzyloxy)-7-chloro-5-((4-fluorophenyl)thio)quinoline **73** (275 mg, 0.69 mmol) in
12 dichloromethane (7 mL) at 0°C . Upon completion the reaction was quenched reaction with
13 dimethyl sulfide (70 μL , 1.53 mmol). The crude mixture was poured into saturated sodium
14 bicarbonate, dried over sodium sulfate, decanted and concentrated under reduced pressure. The
15 residue was dissolved in minimal dichloromethane and purified by automated normal phase
16 chromatography eluting with 0-20% ethyl acetate/heptane over a 20 minute gradient. The
17 product attained was 8-(benzyloxy)-7-chloro-5-((4-fluorophenyl)sulfonyl)quinoline **74** (200 mg,
18 67% yield). ^1H NMR (400 MHz, CDCl_3) δ ppm 9.03 - 9.08 (m, 1H) 9.00 (dd, $J = 8.8, 1.5$ Hz,
19 1H) 8.53 - 8.56 (m, 1H) 7.96 - 8.02 (m, 2H) 7.53 - 7.60 (m, 3H) 7.34 - 7.41 (m, 3H) 7.17 - 7.24
20 (m, 2H) 5.61 - 5.66 (m, 2H). MS (ES+) m/z 428.0 $[\text{M}+\text{H}]^+$.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 *Step 6.* 8-(benzyloxy)-7-chloro-5-((4-fluorophenyl)sulfonyl)quinoline **74** (200 mg, 0.470
37 mmol) was dissolved in 6 N aqueous hydrochloric acid (5 mL). 1,4-Dioxane (3 mL) was added
38 to solubilize the reaction. The reaction was heated reaction to 80°C and upon completion cooled
39 to room temperature. The mixture was concentrated under reduced pressure, azeotroped with
40 acetonitrile and suspended in chloroform. The precipitate was collected by vacuum filtration and
41 dried under high vacuum. The product attained was 7-chloro-5-((4-
42 fluorophenyl)sulfonyl)quinolin-8-ol **19** (135 mg, 86% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ
43 ppm 9.00 (d, $J = 4.11$ Hz, 1 H) 8.91 (d, $J = 8.72$ Hz, 1 H) 8.46 (s, 1 H) 8.11 - 8.18 (m, 2 H) 7.78
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(dd, $J = 8.72, 4.23$ Hz, 1 H) 7.43 (t, $J = 8.05$ Hz, 2 H). ^{19}F NMR (376 MHz, DMSO- d_6) δ ppm -199.76 (s, 1 F). MS (ES+) m/z 337.9 $[\text{M}+\text{H}]^+$.

7-Fluoro-5-((4-fluorophenyl)sulfonyl)quinolin-8-ol (20). *Step 1.* 4-fluorobenzenethiol (4.8 mL, 44.7 mmol) was added to a solution mixture of 8-(benzyloxy)-7-fluoro-5-iodoquinoline **42** (11.3 g, 29.8 mmol), tris(dba)dipalladium(0) (1.3 g, 1.5 mmol), Xantphos (862 mg, 1.4 mmol) in 1,4-dioxane (132 mL) at room temperature followed by addition of cesium carbonate (29.1 g, 89.4 mmol). The mixture was heated to 90°C under nitrogen. Upon completion, the mixture was poured into brine, extracted with ethyl acetate, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude material was dissolved in a minimal quantity of dichloromethane and purified by automated normal phase chromatography (0-30% ethyl acetate/heptane). The product-containing fractions were combined to give 8-(benzyloxy)-7-fluoro-5-((4-fluorophenyl)thio)quinoline **75** (10.8 g, 95% yield). ^1H NMR (400 MHz, DMSO- d_6) δ ppm 9.05 (dd, $J = 4.20, 1.48$ Hz, 1 H) 8.63 (dd, $J = 8.59, 1.58$ Hz, 1 H) 7.60 - 7.74 (m, 2 H) 7.46 - 7.53 (m, 2 H) 7.30 - 7.42 (m, 6 H) 7.16 - 7.25 (m, 2 H) 5.50 (s, 2 H). MS (ES+) m/z 380.0 $[\text{M}+\text{H}]^+$.

Step 2. 3-chlorobenzoperoxoic acid (12.7 g, 56.7 mmol) was added to a solution 8-(benzyloxy)-7-fluoro-5-((4-fluorophenyl)thio)quinoline **75** (10.8 g, 28.3 mmol) in dichloromethane (57 mL) at room temperature. The reaction mixture was filtered, solids discarded, filtrate washed with 10% sodium sulfite solution, followed by saturated sodium bicarbonate and brine. The solution was dried over sodium sulfate, decanted and concentrated under reduced pressure. The crude material was dissolved in minimal dichloromethane and

1
2
3 purified by automated normal phase chromatography (0-100% ethyl acetate/heptane) to provide
4 9.0 g (77 % yield) of 8-(benzyloxy)-7-fluoro-5-((4-fluorophenyl)sulfonyl)quinoline **76**. ¹H NMR
5 (400 MHz, DMSO-*d*₆) δ ppm 9.08 (dd, *J* = 4.11, 1.33 Hz, 1 H) 8.88 - 8.99 (m, 1 H) 8.50 (d, *J* =
6 10.67 Hz, 1 H) 8.17 (dd, *J* = 8.84, 5.05 Hz, 2 H) 7.73 (dd, *J* = 8.81, 4.14 Hz, 1 H) 7.25 - 7.57 (m,
7 7 H) 5.62 (s, 2 H). MS (ES+) *m/z* 412.0 [M+H]⁺.
8
9
10
11
12
13
14

15 *Step 3.* 8-(benzyloxy)-7-fluoro-5-((4-fluorophenyl)sulfonyl)quinoline **76** (9.0 g, 21.9
16 mmol) was suspended in 6 N aqueous hydrochloric acid (120 mL). 1-4-Dioxane (40 mL) was
17 added to solubilize reaction. The mixture was heated to 90°C. Upon completion, the flask was
18 cooled to room temperature, concentrated under reduced pressure and suspended in toluene. The
19 solids were collected via vacuum, filtration. The solids were dissolved in chloroform, poured
20 into 1N sodium hydroxide solution, extracted with chloroform, dried over magnesium sulfate,
21 filtered and concentrated under reduced pressure. The product, 7-fluoro-5-((4-
22 fluorophenyl)sulfonyl)quinolin-8-ol **20** (5.9 g, 84% yield) was isolated as the free base. ¹H
23 NMR (400 MHz, DMSO-*d*₆) δ ppm 8.99 (dd, *J* = 4.17, 1.39 Hz, 1 H) 8.91 (dd, *J* = 8.84, 1.45
24 Hz, 1 H) 8.46 (d, *J* = 10.93 Hz, 1 H) 8.13 (dd, *J* = 8.87, 5.08 Hz, 2 H) 7.72 (dd, *J* = 8.78, 4.17
25 Hz, 1 H) 7.43 (t, *J* = 8.81 Hz, 2 H). MS (ES+) *m/z* 322.0 [M+H]⁺.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **7-Fluoro-5-tosylquinolin-8-ol (21).** *Step 1.* A mixture of 8-benzyloxy-7-fluoro-5-iodo-
46 quinoline **42** (3.0 g, 7.9 mmol), 4-methylbenzenethiol (1.4 g, 11.8 mmol), tris(dba)dipalladium
47 (362 mg, 0.40 mmol), Xantphos (228 mg, 0.40 mmol) and cesium carbonate (7.7 g, 23.7 mmol)
48 in dioxane (5 mL) was degassed with nitrogen. The resultant mixture was heated at 90°C
49 overnight. The reaction mixture was filtered through a plug of silica gel eluting with ethyl
50
51
52
53
54
55
56
57
58
59
60

1
2
3 acetate. The filtrate was concentrated and purified automated normal-phase chromatography
4
5 eluting 0-30% ethyl acetate/hexanes to yield 2.4 g (82%) of 8-benzyloxy-7-fluoro-5-(p-
6
7 tolylsulfanyl)quinoline **77**. ^1H NMR (400 MHz, CDCl_3) δ ppm 9.01 (dd, $J = 4.04, 1.52$ Hz, 1 H)
8
9 8.62 - 8.67 (m, 1 H) 7.52 - 7.58 (m, 2 H) 7.44 (dd, $J = 8.59, 4.29$ Hz, 1 H) 7.29 - 7.39 (m, 4 H)
10
11 7.09 - 7.20 (m, 4 H) 5.55 (s, 2 H) 2.34 (s, 3 H). MS (ES+) m/z 376.1 $[\text{M}+\text{H}]^+$.
12
13
14

15 *Step 2.* To a mixture of 8-benzyloxy-7-fluoro-5-(p-tolylsulfanyl)quinoline **77** (2.4 g, 6.4
16
17 mmol) in DCM (40 mL) at 0°C was added 3-chlorobenzenecarboperoxoic acid (2.2 g, 12.9
18
19 mmol). The reaction was stirred at 0°C for 1 hour. The cooling bath was removed and
20
21 additional 0.5 g of mCPBA was added to drive the reaction to completion. Sodium sulfite (10%
22
23 solution, 75 mL) was added and stirred for 60 minutes then removed followed by sodium
24
25 hydroxide aqueous solution (1 N, 75 mL). The solution was stirred at room temperature
26
27 overnight. The aqueous phase was removed and organic solution was dried over sodium
28
29 sulfate. The residue was purified by normal phase chromatography eluting 0-50% ethyl
30
31 acetate/hexanes. The material was taken on as is without additional purification (oil contained
32
33 residual ethyl acetate). Isolated 2.4 g (92% yield) of 8-benzyloxy-7-fluoro-5-(p-
34
35 tolylsulfonyl)quinoline **78** as a viscous, yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 8.95 -
36
37 9.03 (m, 2 H) 8.33 (d, $J = 11.12$ Hz, 1 H) 7.79 - 7.86 (m, 2 H) 7.47 - 7.54 (m, 3 H) 7.29 - 7.39
38
39 (m, 5 H) 5.67 (s, 2 H) 2.40 (s, 3 H). MS (ES+) m/z 408.0 $[\text{M}+\text{H}]^+$.
40
41
42
43
44
45

46 *Step 3.* A solution of 8-benzyloxy-7-fluoro-5-(p-tolylsulfonyl)quinoline **78** (2.4 g, 5.9
47
48 mmol) and 6 M aqueous hydrochloric acid (30 mL, 180 mmol) in dioxane (30 mL) to aid
49
50 solubility, was heated to 90°C for 3 hours. The solution was cooled to ambient temperature and
51
52 neutralized with 10% sodium hydroxide solution. Excess dioxane was removed by rotary
53
54 evaporator and solids formed. The aqueous mixture was neutralized with 10% aqueous sodium
55
56
57
58
59
60

1
2
3 hydroxide solution. The orange solids were collected by filtration and washed with water to
4
5 provide the title compound **21** (1.7 g, 89% yield). ¹H NMR (400 MHz, CDCl₃) δ ppm 9.04 (dd,
6
7 *J* = 8.72, 1.39 Hz, 1 H) 8.86 (dd, *J* = 4.29, 1.52 Hz, 1 H) 8.42 (d, *J* = 10.36 Hz, 1 H) 7.82 - 7.87
8
9 (m, 2 H) 7.56 (dd, *J* = 8.72, 4.17 Hz, 1 H) 7.30 (d, *J* = 8.08 Hz, 2 H) 2.39 (s, 3 H). MS (ES+)
10
11 *m/z* 318.0 [M+H]⁺.
12
13
14
15
16
17

18 **7-Chloro-5-tosylquinolin-8-ol (22)**. *Step 1*. 1,4-Dioxane was degassed by sparging with
19
20 nitrogen for 10 minutes prior to initiating reaction. A solution of 8-(benzyloxy)-7-chloro-5-((2-
21
22 (trimethylsilyl)ethyl)thio)quinoline **72** (2.1 g, 5.2 mmol) in 1,4-dioxane (6 mL) was added to a
23
24 250 mL round bottom flask charged with a magnetic stir bar, 4-iodotoluene (1.3 g, 5.75 mmol),
25
26 tris(dba)dipalladium(0) (239 mg, 0.26 mmol), Xantphos (151 mg, 0.26 mmol). To this solution
27
28 mixture was added cesium carbonate (5.1 g, 15.7 mmol), followed by tetrabutylammonium
29
30 fluoride (6.3 mL, 1M THF, 6.3 mmol). The reaction was sealed reaction with rubber septum and
31
32 heated to 90°C. Upon completion the mixture was cooled to room temperature, poured into
33
34 brine, extracted with ethyl acetate, dried over sodium sulfate, filtered, and concentrated under
35
36 reduced pressure. The crude mixture was dissolved in minimal dichloromethane and purified by
37
38 automated normal phase chromatography eluting with 0-40% ethyl acetate/heptane gradient over
39
40 35 minutes. The product attained was 8-(benzyloxy)-7-chloro-5-(p-tolylthio)quinoline **79** (1.7 g,
41
42 81% yield) was taken on directly in the next step. MS (ES+) *m/z* 392.0 [M+H]⁺.
43
44
45
46
47
48

49 *Step 2*. 3-Chlorobenzoperoxoic acid (2.0 g, 8.9 mmol) was added to a solution of 8-
50
51 (benzyloxy)-7-chloro-5-(p-tolylthio)quinoline **79** (1.6 g, 4.0 mmol) in dichloromethane (8.7 mL)
52
53 at 0 °C. The reaction was warmed to room temperature. Benzoic acid was collected by filtration,
54
55 the filtrate was washed with 10% sodium sulfite solution, followed by saturated sodium
56
57
58
59
60

1
2
3 bicarbonate and brine. The solution was dried over sodium sulfate, decanted and concentrated
4 under reduced pressure. The residue was dissolved in a minimal quantity of dichloromethane
5 and purified by automated normal phase chromatography (12 g silica gel cartridge) eluting with
6 0-100% ethyl acetate/heptane. The product attained was 8-(benzyloxy)-7-chloro-5-
7 tosylquinoline **80** (1.1 g, 64% yield). MS (ES+) m/z 424.0 $[M+H]^+$.
8
9
10
11
12
13
14

15 *Step 3.* 8-(benzyloxy)-7-chloro-5-tosylquinoline **80** (1.1 g, 2.59 mmol) was suspended in
16 of 6 N aqueous hydrochloric acid (5 mL) followed by addition of 1,4-dioxane (6 mL) to
17 solubilize reaction and resultant mixture heated to 65°C. Upon completion the reaction was
18 cooled to room temperature, concentrated under reduced pressure and azeotroped with
19 acetonitrile. The material was taken up in chloroform, product precipitated and solids collected
20 via vacuum filtration. The product attained was 7-chloro-5-tosylquinolin-8-ol **22** (600 mg, 69%
21 yield) as a light yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ ppm 8.98 (dd, $J = 4.17, 1.39$
22 Hz, 1 H) 8.89 (dd, $J = 8.78, 1.39$ Hz, 1 H) 8.42 (s, 1 H) 7.91 (d, $J = 8.34$ Hz, 2 H) 7.77 (dd, $J =$
23 8.78, 4.23 Hz, 1 H) 7.39 (d, $J = 8.15$ Hz, 2 H) 2.33 (s, 3 H). MS (ES+) m/z 334.0 $[M+H]^+$.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 **7-Bromo-5-tosylquinolin-8-ol (23).** *Step 1.* To a slurry of 8-((4-methoxybenzyl)oxy)-5-
41 tosylquinoline **35** (500 mg, 1.2 mmol) in dichloromethane was added trifluoroacetic acid (92 μL ,
42 1.2 mmol). The reaction was maintained at ambient temperature for 1 hour prior to concentration
43 to an oily residue. Water was added and a gooey solid formed. The aqueous solution was
44 neutralized with 6 M sodium hydroxide and diluted with dichloromethane. The layers were
45 separated and organic solution dried over sodium sulfate. Concentration gave a sticky solid that
46 was purified by automated normal-phase chromatography using 0-10%
47 methanol/dichloromethane as an eluent to provide 420 mg (85% yield) of 5-(p-
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Tolylsulfonyl)quinolin-8-ol **7**. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 8.94 (dd, $J = 4.04, 1.52$
4 Hz, 1 H) 8.88 (dd, $J = 8.84, 1.52$ Hz, 1 H) 8.39 (d, $J = 8.34$ Hz, 1 H) 7.83 (d, $J = 8.34$ Hz, 2 H)
5
6 7.72 (dd, $J = 8.72, 4.17$ Hz, 1 H) 7.37 (d, $J = 8.34$ Hz, 2 H) 7.27 (d, $J = 8.34$ Hz, 1 H) 3.34 (br.
7
8 s., 1 H) 2.32 (s, 3 H). MS (ES+) m/z 301.0 $[\text{M}+\text{H}]^+$.
9
10
11

12
13 *Step 2.* A solution of 5-(p-tolylsulfonyl)quinolin-8-ol **7** (290 mg, 0.97 mmol) and N-
14
15 bromosuccinimide (206 mg, 1.1 mmol) in chloroform was vigorously stirred at 40°C for 3 hours.
16
17 The resulting solution was washed with 10% aqueous sodium thiosulfate solution (1 X 10 mL)
18
19 and layers separated. The solvent was removed by rotary evaporator and product isolated as a
20
21 yellow-brown solid. The solids were suspended in dichloromethane and diethyl ether, material
22
23 collected by filtration and washed with ether to yield pale yellow solids. The filtrate was
24
25 collected and concentrated to a residue to provide 7-bromo-5-(p-tolylsulfonyl)quinolin-8-ol **23**
26
27 (131 mg, 36% yield). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 8.96 (d, $J = 4.04$ Hz, 1 H) 8.88 (d,
28
29 $J = 7.83$ Hz, 1 H) 8.51 (s, 1 H) 7.91 (d, $J = 8.34$ Hz, 2 H) 7.77 (dd, $J = 8.59, 4.29$ Hz, 1 H) 7.39
30
31 (d, $J = 8.59$ Hz, 2 H) 2.33 (s, 3 H). MS (ES+) m/z 379.1 $[\text{M}+\text{H}]^+$.
32
33
34
35
36
37
38
39

40 **5-Tosyl-7-(trifluoromethyl)quinolin-8-ol (24).** *Step 1.* A mixture of 2-fluoro-3-
41
42 (trifluoromethyl)aniline (1.8 mL, 13.9 mmol), glycerol (2.5 mL, 34.8 mmol) and sodium 3-
43
44 nitrobenzenesulfonate (3.7 g, 16.7 mmol) was treated with 70% aqueous sulfuric acid (12 mL,
45
46 225.1 mmol) and heated to reflux (140°C) for 2.5 hours. The mixture was cooled to ambient
47
48 temperature, diluted with water (25 mL) and basified with 50% sodium hydroxide aqueous
49
50 solution. Filtration through Celite captured a solid which was washed with
51
52 dichloromethane. The aqueous filtrate was extracted with dichloromethane and combined
53
54 extracts evaporated to give an oily residue. The oily residue and brown solids were combined and
55
56
57
58
59
60

1
2
3 purified by automated normal phase chromatography (50 g silica gel cartridge) eluting with 0-
4 50% ethyl acetate/heptane. Isolated a pale yellow solid (2.1 g, 70% yield) as 8-fluoro-7-
5 (trifluoromethyl)quinoline **81**. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ ppm 9.08 - 9.15 (m, 1 H) 8.60
6 (dd, $J = 8.46, 1.39$ Hz, 1 H) 8.06 (d, $J = 8.59$ Hz, 1 H) 7.89 (t, $J = 7.58$ Hz, 1 H) 7.83 (dd, $J =$
7 8.34, 4.04 Hz, 1 H). MS (ES+) m/z 216.2 $[\text{M}+\text{H}]^+$.

8
9
10
11
12
13
14
15 *Step 2.* To a suspension of sodium hydride (706 mg, 17.6 mmol) in THF (20 mL) was
16 added 2-(trimethylsilyl)ethanol (2.5 mL, 17.6 mmol). The resultant slurry was stirred for 15
17 minutes at ambient temperature. The this suspension was added to a solution of 8-fluoro-7-
18 (trifluoromethyl)quinoline **81** (1.0 g, 4.6 mmol) in THF (5 mL) and stirred for 1 hour. The
19 reaction mixture was treated with ice and partitioned between water and ethyl acetate. The
20 layers were separated and the aqueous layer extracted ethyl acetate (1 X 20 mL). The organic
21 washes were combined, extracted with brine (1 X 20 mL) and dried over sodium
22 sulfate. Concentration yielded a pale yellow oil that was purified by automated normal phase
23 chromatography (25 g) eluting 0-40% ethyl acetate/heptane. Trimethyl-[2-[[7-(trifluoromethyl)-
24 8-quinolyl]oxy]ethyl]silane **82** was isolated as a pale yellow oil (1.4 g, 99% yield). $^1\text{H NMR}$
25 (400 MHz, CDCl_3) δ ppm 8.94 - 9.05 (m, 1 H) 8.21 (d, $J = 8.34$ Hz, 1 H) 7.65 - 7.76 (m, 1 H)
26 7.61 (d, $J = 8.59$ Hz, 1 H) 7.52 (dd, $J = 8.21, 4.17$ Hz, 1 H) 4.59 - 4.73 (m, 2 H) 1.34 - 1.47 (m,
27 3 H) 0.04 - 0.12 (m, 9 H). MS (ES+) m/z 314.0 $[\text{M}+\text{H}]^+$.

28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46 *Step 3.* To a solution of trimethyl-[2-[[7-(trifluoromethyl)-8-quinolyl]oxy]ethyl]silane **82**
47 (500 mg, 1.6 mmol) dissolved in 10 mL dioxane was added 37% hydrochloric acid (1.2 mL).
48 The reaction allowed to stir over the 2 days (for convenience) at ambient temperature. The
49 reaction mixture was concentrated to a pale yellow solid. Assumed quantitative conversion to
50 provide 7-(trifluoromethyl)quinolin-8-ol hydrochloride **83**. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ
51
52
53
54
55
56
57
58
59
60

1
2
3 ppm 8.99 (dd, $J = 4.17, 1.64$ Hz, 1 H) 8.47 (dd, $J = 8.34, 1.52$ Hz, 1 H) 7.74 (dd, $J = 8.34, 4.29$
4 Hz, 1 H) 7.68 (d, $J = 8.59$ Hz, 1 H) 7.54 (d, $J = 8.84$ Hz, 1 H). MS (ES+) m/z 214.0 [M+H]⁺.
5
6
7

8 *Step 4.* 5-Iodo-7-(trifluoromethyl)quinolin-8-ol **84** was prepared in a similar manner as
9 Compound 18, step 2. The product was prepared from 7-(trifluoromethyl)quinolin-8-ol
10 hydrochloride **83** (400 mg, 1.6 mmol) providing 543 mg (99% yield). ¹H NMR (400 MHz,
11 DMSO-*d*₆) δ ppm 9.00 (dd, $J = 4.17, 1.39$ Hz, 1 H) 8.39 (dd, $J = 8.59, 1.52$ Hz, 1 H) 8.14 (s, 1
12 H) 7.86 (dd, $J = 8.59, 4.04$ Hz, 1 H). MS (ES+) m/z 340.0 [M+H]⁺.
13
14
15
16
17
18
19

20 *Step 5.* To a suspension of 5-iodo-7-(trifluoromethyl)quinolin-8-ol **84** (540 mg, 1.5
21 mmol) in acetonitrile (10 mL) and DMF (5 mL) was added potassium carbonate (242 mg, 1.7
22 mmol). To this mixture was added 4-methoxybenzyl chloride (0.26 mL, 1.9 mmol) dropwise
23 followed by catalytic sodium iodide. The reaction was allowed to stir at ambient temperature
24 overnight. The reaction was heated to 50°C for 3 hours. The reaction was cooled to ambient
25 temperature and filtered. The filtrate was concentrated to an oil and partitioned between ethyl
26 acetate and brine. The layers were separated and ethyl acetate layer was dried over sodium
27 sulfate. Purification was accomplished by automated normal phase chromatography (25 g silica
28 gel cartridge) eluting 0-35% ethyl acetate/heptane to yield 429 mg (58% yield) of 5-iodo-8-[(4-
29 methoxyphenyl)methoxy]-7-(trifluoromethyl)quinoline **85** as a waxy orange solid. ¹H NMR
30 (400 MHz, DMSO-*d*₆) δ ppm 9.12 (dd, $J = 4.04, 1.52$ Hz, 1 H) 8.47 (dd, $J = 8.72, 1.64$ Hz, 1 H)
31 8.29 (s, 1 H) 7.87 (dd, $J = 8.72, 4.17$ Hz, 1 H) 7.40 - 7.50 (m, 2 H) 6.90 - 7.01 (m, 2 H) 5.56 (s, 2
32 H) 3.72 - 3.80 (m, 3 H). MS (ES+) m/z 482.0 [M+Na]⁺.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

51 *Step 6.* 8-[(4-Methoxyphenyl)methoxy]-5-(*p*-tolylsulfanyl)-7-(trifluoromethyl)quinoline
52 **86** was prepared in a similar manner as Compound 18 step 4. The product was prepared from 5-
53 iodo-8-[(4-methoxyphenyl)methoxy]-7-(trifluoromethyl)quinoline **85** (200 mg, 0.43 mmol) to
54
55
56
57
58
59
60

1
2
3 yield 162 mg (81% yield). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 9.16 (dd, $J = 4.17, 1.64$ Hz, 1
4 H) 8.71 (dd, $J = 8.72, 1.64$ Hz, 1 H) 7.76 - 7.86 (m, 2 H) 7.43 - 7.51 (m, 2 H) 7.13 - 7.23 (m, 4
5 H) 6.93 - 7.01 (m, 2 H) 5.60 (s, 2 H) 3.74 - 3.81 (m, 3 H) 2.27 (s, 3 H). MS (ES+) m/z 478.0
6
7
8
9
10 $[\text{M}+\text{Na}]^+$.

11
12
13 *Step 7.* To a mixture of 8-[(4-methoxyphenyl)methoxy]-5-(p-tolylsulfanyl)-7-
14 (trifluoromethyl)quinoline **86** (160 mg, 0.35 mmol) in DCM (5 mL) at ambient temperature was
15 added 3-chlorobenzenecarboperoxoic acid (173 mg, 0.70 mmol). The reaction was stirred for 2
16 hours. The reaction was quenched by addition of dimethyl sulfide. The mixture was extracted
17 with 10% sodium sulfite aqueous solution. The organic layer was dried over sodium sulfate and
18 concentrated in vacuo. Purification was accomplished by automated reversed phase
19 chromatography eluting 20-95% ACN/water with 0.05% TFA as modifier. Isolated 5-(p-
20 tolylsulfonyl)-7-(trifluoromethyl)quinolin-8-ol **24** (109 mg, 85% yield) as a solid. ^1H NMR (400
21 MHz, $\text{DMSO-}d_6$) δ ppm 9.02 (d, $J = 4.29$ Hz, 1 H) 8.94 (d, $J = 8.84$ Hz, 1 H) 8.48 (s, 1 H) 7.86 -
22 7.96 (m, 3 H) 7.39 (d, $J = 8.34$ Hz, 2 H) 2.34 (s, 3 H). MS (ES+) m/z 368.0 $[\text{M}+\text{H}]^+$.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 **5-(Cyclopentylsulfonyl)-7-fluoroquinolin-8-ol (25).** *Step 1.* A mixture of 8-benzyloxy-
41 7-fluoro-5-iodo-quinoline **42** (10.4 g, 27.4 mmol), cyclopentanethiol (4.4 mL, 41.1
42 mmol), tris(dba)dipalladium (1.2 g, 1.3 mmol), Xantphos (0.8 g, 1.3 mmol) and cesium
43 carbonate (26.8 g, 82.2 mmol) in dioxane (100 mL) was degassed with nitrogen for 15
44 minutes. The resultant mixture was heated at 90°C for 3 hours. The mixture was passed through
45 a plug of silica eluting with ethyl acetate and concentrated to a residue. The residue was purified
46 via silica gel chromatography eluting with ethyl acetate/hexanes (0-30%) to give 8-benzyloxy-5-
47 cyclopentylsulfanyl-7-fluoro-quinoline **87** (9.2 g, 95% yield) as a slightly colored oil. ^1H NMR
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 (400 MHz, CDCl₃) δ ppm 9.01 (dd, *J* = 4.2, 1.6 Hz, 1 H) 8.72 (dd, *J* = 8.6, 1.8 Hz, 1 H) 7.52 -
4 7.60 (m, 2 H) 7.41 - 7.52 (m, 2 H) 7.30 - 7.40 (m, 3 H) 5.48 - 5.55 (m, 2 H) 3.51 - 3.62 (m, 1 H)
5
6 1.95 - 2.10 (m, 2 H) 1.74 - 1.89 (m, 2 H) 1.56 - 1.70 (m, 4 H). MS (ES+) *m/z* 354.0 [M+H]⁺.
7
8
9

10 *Step 2.* To a stirring solution of 8-benzyloxy-5-cyclopentylsulfanyl-7-fluoro-quinoline **87**
11 (9.2 g, 26.1 mmol) in DCM (200 mL), 3-chlorobenzenecarboperoxoic acid (13.5 g, 54.9 mmol)
12 was added. The solution was stirred at ambient temperature for 1 hour. The reaction was
13 quenched by addition of dimethyl sulfide (20 drops). The resultant mixture was stirred for 20
14 minutes. Sodium sulfite (1 N solution, 200 mL) was added. The organic solution extracted and
15 separated. The organic layer was dried over sodium sulfate and concentrated. The residue was
16 purified by normal phase chromatography (110 g silica gel cartridge) eluting with ethyl
17 acetate/hexanes (0-50%) to give 8-benzyloxy-5-cyclopentylsulfonyl-7-fluoro-quinoline **88** (7.8
18 g, 77% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 9.06 - 9.15 (m, 2 H) 8.15 (d,
19 *J* = 10.9 Hz, 1 H) 7.59 (dd, *J* = 8.6, 4.3 Hz, 1 H) 7.52 (dd, *J* = 7.7, 1.4 Hz, 2 H) 7.29 - 7.39 (m,
20 3H) 5.70 (s, 2 H) 3.61 (quin, *J* = 7.6 Hz, 1 H) 2.01 - 2.16 (m, 2 H) 1.73 - 1.90 (m, 4 H) 1.56 -
21 1.68 (m, 2 H). MS (ES+) *m/z* 386.0 [M+H]⁺.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37

38 *Step 3.* 8-benzyloxy-5-cyclopentylsulfonyl-7-fluoro-quinoline **88** (7.8 g, 20.3 mmol) in
39 hydrochloric acid (6 N, 150 mL, 900 mmol) was stirred at 100°C for two hours. The solution
40 was cooled in ice-water bath for 30 minutes. The yellow precipitate was isolated by filtration
41 and washed with diethyl ether and air-dried to yield a green solid. The solid was free-based by
42 dissolving it in a slightly basic solution (0.05 N NaOH, 20 mL) then the solution was adjusted to
43 slightly acidic by adding hydrochloric acid (1 N). The solution was extracted with
44 dichloromethane (3 X 100 mL). The combined organic solution was washed with brine (2 X 100
45 mL) and dried over sodium sulfate. The solution was filtered and concentrated to give slightly
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 colored solid. The solid was purified further by crystallization in heptane/chloroform to give 5-
4 cyclopentylsulfonyl-7-fluoro-quinolin-8-ol **25** (4.3 g, 72% yield) as a solid. ¹H NMR (400 MHz,
5 DMSO-*d*₆) δ ppm 8.96 - 9.07 (m, 2 H) 8.09 - 8.18 (m, 1 H) 7.80 (dd, *J* = 8.7, 4.2 Hz, 1 H) 3.81 -
6 3.94 (m, 1 H) 1.82 - 1.97 (m, 2 H) 1.61 - 1.82 (m, 4 H) 1.47 - 1.60 (m, 2 H). MS (ES+) *m/z*
7 296.0 [M+H]⁺.
8
9
10
11
12
13
14
15
16
17

18 **7-Chloro-5-(cyclopentylsulfonyl)quinolin-8-ol (26).** *Step 1.* To a stirring solution of 8-
19 benzyloxy-5-bromo-7-chloro-quinoline **71** (7.6 g, 21.8 mmol) in dioxane (100 mL),
20 cyclopentanethiol (3.5 mL, 32.7 mmol), tris(*dba*)dipalladium (1.0 g, 1.0 mmol), Xantphos (0.63
21 g, 1.0 mmol), cesium carbonate (21.3 g, 65.4 mmol) were added. Nitrogen was bubbled in for 3
22 minutes and the resultant solution heated at 90°C overnight. Water (150 mL) was added. The
23 solution was extracted with ethyl acetate (3 X 100 mL). The combined organic solution was
24 extracted with brine (50 mL) and dried over sodium sulfate. The organic solution was filtered
25 and concentrated. The residue was purified by automated normal phase chromatography and
26 eluted with ethyl acetate/hexane (0-30%) to give 8-benzyloxy-7-chloro-5-cyclopentylsulfanyl-
27 quinoline **89** (7.5 g, 93% yield) as a slightly colored oil. ¹H NMR (400 MHz, CDCl₃) δ ppm
28 9.01 (dd, *J* = 4.2, 1.6 Hz, 1 H) 8.71 - 8.80 (m, 1 H) 7.66 - 7.69 (m, 1 H) 7.60 - 7.66 (m, 2 H) 7.46
29 - 7.54 (m, 1 H) 7.31 - 7.44 (m, 3 H) 5.45 - 5.52 (m, 2 H) 3.52 - 3.63 (m, 1H) 1.96 - 2.10 (m, 2 H)
30 1.76 - 1.91 (m, 2 H) 1.56 - 1.72 (m, 4 H). MS (ES+) *m/z* 370.0 [M+H]⁺.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

48 *Step 2.* To a stirring solution of 8-benzyloxy-7-chloro-5-cyclopentylsulfanyl-quinoline
49 **89** (7.5 g, 20.4 mmol) in DCM (100 mL) at 0°C, 3-chlorobenzenecarboperoxoic acid (10.0 g,
50 40.8 mmol) was added slowly into the solution. The reaction was continued at 0°C for 20
51 minutes then at room temperature for 20 additional minutes. Sodium sulfite (1.0 N solution, 30
52
53
54
55
56
57
58
59
60

mL) was added. Sodium hydroxide solution (1.0 N, 100 mL) was added. The solution was stirred at room temperature for 20 minutes then the reaction was diluted with DCM (100 mL). The aqueous phase was discarded. The organic solution was extracted with sodium hydroxide solution (1 N, 50 mL), water (100 mL), brine (100 mL) and dried over sodium sulfate. The solution was filtered and concentrated. The residue was purified by automated normal-phase chromatography and eluted with ethyl acetate/hexane (0-60%) to give 8-benzyloxy-7-chloro-5-cyclopentylsulfonyl-quinoline **90** (6.9 g, 84% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ ppm 9.06 - 9.18 (m, 2 H) 8.35 (s, 1 H) 7.54 - 7.67 (m, 3 H) 7.32 - 7.43 (m, 3 H) 5.69 (s, 2 H) 3.56 - 3.68 (m, 1 H) 2.06 - 2.19 (m, 2 H) 1.77 - 1.91 (m, 4 H) 1.60 - 1.71 (m, 2 H). MS (ES+) m/z 402.0 $[\text{M}+\text{H}]^+$.

Step 3. 8-benzyloxy-7-chloro-5-cyclopentylsulfonyl-quinoline **90** (6.9 g, 17.1 mmol) in aqueous hydrochloric acid (6 N, 81 mL, 489 mmol) was stirred at 100°C for two hours. The solution was cooled in ice-water bath for 30 min. The yellow precipitate was isolated by filtration and washed with diethyl ether and air-dried to give 7-chloro-5-cyclopentylsulfonyl-quinolin-8-ol **26** (5.2 g, 98% yield) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 8.99 - 9.11 (m, 2 H) 8.14 (s, 1 H) 7.85 (dd, $J = 8.6, 4.0$ Hz, 1 H) 3.82 - 3.93 (m, 1 H) 1.82 - 1.94 (m, 2 H) 1.62 - 1.82 (m, 4 H) 1.49 - 1.61 (m, 2 H). MS (ES+) m/z 312.0 $[\text{M}+\text{H}]^+$.

5-(Cyclopentylsulfonyl)-7-(trifluoromethyl)quinolin-8-ol (27). *Step 1.* 5-Cyclopentylsulfonyl-8-[(4-methoxyphenyl)methoxy]-7-(trifluoromethyl)quinoline **91** was prepared in a similar manner to Compound 18, step 2. The product was prepared from 5-iodo-8-[(4-methoxyphenyl)methoxy]-7-(trifluoromethyl)quinoline **85** (215 mg, 0.46 mmol) as described in compound 24, step 5 to yield 183 mg (90% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm

1
2
3 9.16 (dd, $J = 4.04, 1.52$ Hz, 1 H) 8.81 (dd, $J = 8.72, 1.64$ Hz, 1 H) 7.78 - 7.87 (m, 2 H) 7.43 -
4
5 7.51 (m, 2 H) 6.92 - 7.00 (m, 2 H) 5.55 (s, 2 H) 3.76 - 3.79 (m, 3 H) 3.70 - 3.76 (m, 1 H) 1.99
6
7 (dd, $J = 12.63, 5.56$ Hz, 2 H) 1.70 - 1.81 (m, 2 H) 1.48 - 1.65 (m, 4 H). MS (ES+) m/z 456.0
8
9 [M+Na]⁺.
10
11

12
13 *Step 2.* 5-Cyclopentylsulfonyl-7-(trifluoromethyl)quinolin-8-ol **27** was prepared in a
14
15 similar manner to Compound 24, step 7. The product was prepared from 5-cyclopentylsulfanyl-
16
17 8-[(4-methoxyphenyl)methoxy]-7-(trifluoromethyl)quinoline **91** (180 mg, 0.41 mmol) to provide
18
19 97 mg (67% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.11 (dd, $J = 6.69, 1.14$ Hz, 2 H)
20
21 8.20 (s, 1 H) 7.94 - 8.01 (m, 1 H) 3.88 (s, 1 H) 1.84 - 1.95 (m, 2 H) 1.73 - 1.83 (m, 2 H) 1.63 -
22
23 1.72 (m, 2 H) 1.49 - 1.60 (m, 2 H). MS (ES+) m/z 346.0 [M+H]⁺.
24
25
26
27
28
29

30
31 **7-Fluoro-5-(pyrrolidin-1-ylsulfonyl)quinolin-8-ol (28).** *Step 1.* To chlorosulfonic acid
32
33 (21 mL, 315 mmol) heated to 100⁰C was added 7-fluoroquinolin-8-ol **41** (7.0 g, 42.9 mmol). A
34
35 reflux condenser was affixed and the mixture is stirred at 100⁰C overnight. The mixture is then
36
37 poured on ice (210 g) with care to limit the exotherm. The aqueous layer was extracted with
38
39 chloroform (3 x 200 mL). The combined organic phases were dried over sodium sulfate,
40
41 concentrated under vacuum to give 5.1 g of 7-fluoro-8-hydroxyquinoline-5-sulfonyl chloride **92**
42
43 as a yellow solid (40% as the HCl salt). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.64 (d, $J = 8.34$
44
45 Hz, 1 H) 9.07 - 9.13 (m, 1 H) 8.01 (dd, $J = 8.59, 5.05$ Hz, 1 H) 7.97 (d, $J = 10.86$ Hz, 1 H). MS
46
47 (ES+) m/z 242.8 [M+H]⁺ (sulfonic acid).
48
49
50

51
52 *Step 2.* Triethylamine (5.6 mL, 40.2 mmol) and pyrrolidine (3.3 mL, 40.2 mmol) were
53
54 dissolved in 80 mL of dichloromethane. A suspension of 7-fluoro-5-pyrrolidin-1-ylsulfonyl-
55
56 quinolin-8-ol **92** (2.9 g, 10.0 mmol) in 40 mL of dichloromethane was added slowly at 0⁰C, then
57
58
59
60

1
2
3 the remaining material was dissolved in 40mL of anhydrous THF. The resultant reaction
4
5 mixture was stirred overnight at ambient temperature. The solvents were evaporated and the
6
7 collected green solid was washed with a minimum of DCM (10mL), then ethyl acetate (2 X 10
8
9 mL) followed by isopropyl alcohol (2 X 10 mL). The solid was dried under high vacuum to
10
11 provide 2.9 g (98% yield) of 7-fluoro-5-(pyrrolidin-1-ylsulfonyl)quinolin-8-ol **28**. ¹H NMR (400
12
13 MHz, DMSO-*d*₆) δ ppm 9.06 (dd, *J* = 8.84, 1.52 Hz, 1 H) 9.02 (dd, *J* = 4.04, 1.52 Hz, 1 H) 8.08
14
15 (d, *J* = 11.12 Hz, 1 H) 7.77 (dd, *J* = 8.84, 4.29 Hz, 1 H) 3.16 - 3.23 (m, 4 H) 1.67 - 1.74 (m, 4
16
17 H). MS (ES+) *m/z* 297.2 [M+H]⁺.
18
19
20
21
22
23
24

25 **7-Chloro-5-(pyrrolidin-1-ylsulfonyl)quinolin-8-ol (29)**. *Step 1*. To a suspension of 8-
26
27 fluoroquinoline-5-sulfonyl chloride **32** (2.0 g, 8.1 mmol) in THF (25 mL) was added DIPEA (2.8
28
29 mL, 16.2 mmol) at room temperature. Pyrrolidine (0.67 mL, 8.1 mmol) was added dropwise as a
30
31 solution in THF (5 mL). Separately, to a suspension of sodium hydride (976 mg, 24.4 mmol) in
32
33 THF (15 mL) was added 2-trimethylsilylethanol (3.5 mL, 24.4 mmol). The crude sulfonamide
34
35 solution was added slowly to the suspension of trimethylsilylethanol sodium salt, and the
36
37 resulting mixture was allowed to stir at room temperature for one hour, and was then quenched
38
39 by addition of water. The resulting mixture was extracted with dichloromethane, and the organic
40
41 layers were collected, dried over magnesium sulfate, and evaporated under reduced pressure.
42
43 Purification by automated normal phase chromatography (0-100% ethyl acetate in heptane)
44
45 afforded trimethyl-[2-[5-pyrrolidin-1-ylsulfonyl-8-quinolyl]oxy]ethyl]silane **93** as a white powder
46
47 (2.1 g, 70% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 8.88-9.08 (m, 2H) 8.14 (d, *J* = 8.59
48
49 Hz, 1 H) 7.75 (dd, *J* = 8.84, 4.04 Hz, 1 H) 7.35 (d, *J* = 8.59 Hz, 1 H) 4.21-4.47 (m, 2 H) 3.06-
50
51 3.24 (m, 4 H) 1.59-1.79 (m, 4 H) 1.15-1.33 (m, 2 H) 0.13 (s, 9 H). MS (ES+) *m/z* 379.0 [M+H]⁺.
52
53
54
55
56
57
58
59
60

1
2
3 *Step 2.* To a solution of trimethyl-[2-[(5-pyrrolidin-1-ylsulfonyl-8-
4 quinolyl)oxy]ethyl]silane **93** (2.1 g, 5.6 mmol) dissolved in THF (35 mL) was added
5
6 tetrabutylammonium fluoride (5.6 mL, 5.6 mmol) as a 1M solution in THF. The mixture was
7
8 allowed to stir for one hour. Solvent was removed under reduced pressure and the resultant
9
10 residue was partitioned between water and dichloromethane. After extracting the aqueous
11
12 mixture, the organic layers were dried over sodium sulfate and concentrated to an oil. Automated
13
14 normal phase silica chromatography (0-10% methanol in dichloromethane) afforded 5-
15
16 Pyrrolidin-1-ylsulfonylquinolin-8-ol **94** (548 mg, 34% yield) as a white solid. ¹H NMR (400
17
18 MHz, DMSO-*d*₆) δ ppm 11.02 (br. s., 1 H) 9.04 (dd, *J* = 8.72, 1.64 Hz, 1 H) 8.98 (dd, *J* = 3.79,
19
20 1.26 Hz, 1 H) 8.09 (d, *J* = 8.34 Hz, 1 H) 7.78 (dd, *J* = 8.84, 4.29 Hz, 1 H) 7.20 (d, *J* = 8.34 Hz, 1
21
22 H) 3.07-3.23 (m, 4 H) 1.71 (dt, *J* = 6.51, 3.44 Hz, 4 H). MS (ES+) *m/z* 279.0 [M+H]⁺.
23
24
25
26
27
28

29 *Step 3.* To a suspension of 5-pyrrolidin-1-ium-1-ylsulfonylquinolin-8-ol **94** (1.3 g, 4.6
30
31 mmol) in chloroform (15 mL) was added N-chlorosuccinimide (620 mg, 4.6 mmol) and the
32
33 resulting mixture was stirred at room temperature overnight. Volatiles were evaporated under
34
35 reduced pressure, and the resulting residue was purified by preparative HPLC (10-100%
36
37 acetonitrile in water with 0.05% trifluoroacetic acid as modifier) to afford 7-Chloro-5-pyrrolidin-
38
39 1-ylsulfonyl-quinolin-8-ol **29** as an orange solid (350 mg, 24% yield). ¹H NMR (400 MHz,
40
41 DMSO-*d*₆) δ ppm 9.12-9.20 (m, 1 H) 8.88-8.95 (m, 1 H) 8.27 (s, 1 H) 7.61-7.68 (m, 1 H) 3.33
42
43 (s, 4 H) 1.86 (s, 4 H). MS (ES+) *m/z* 313.0 [M+H]⁺.
44
45
46
47
48
49
50

51 **7-Methyl-5-(pyrrolidin-1-ylsulfonyl)quinolin-8-ol (30).** *Step 1.* 8-Fluoro-7-methyl-
52
53 quinoline **95** was prepared in a similar manner to Compound 24, step 1. The product was
54
55
56
57
58
59
60

1
2
3 prepared from 2-fluoro-3-methyl-aniline (2.0 g, 15.9 mmol) to provide 2.2 g (88% yield). ¹H
4 NMR (400 MHz, DMSO-*d*₆) δ ppm 8.92 (dd, *J* = 4.17, 1.64 Hz, 1 H) 8.39 (dt, *J* = 8.34, 1.77 Hz,
5 1 H) 7.73 (d, *J* = 8.34 Hz, 1 H) 7.57 (dd, *J* = 8.34, 4.04 Hz, 1 H) 7.51 (dd, *J* = 8.08, 7.07 Hz, 1
6 H) 2.46 (d, *J* = 2.53 Hz, 3 H).
7
8
9
10
11

12
13 *Step 2.* 8-Fluoro-7-methyl-quinoline-5-sulfonyl chloride **96** was prepared in a similar
14 manner to Compound 12, step 1. The product was prepared from 8-fluoro-7-methyl-quinoline
15 **95** (500 mg, 3.1 mmol) to provide 509 mg (63% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm
16 9.48 (dt, *J* = 8.72, 1.71 Hz, 1 H) 9.09 (dd, *J* = 4.80, 1.52 Hz, 1 H) 8.01 (d, *J* = 7.33 Hz, 1 H)
17 7.90 (dd, *J* = 8.72, 4.67 Hz, 1 H) 2.51 - 2.52 (m, 3 H). MS (ES+) *m/z* 260.9 [M+H]⁺.
18
19
20
21
22
23
24

25 *Step 3.* 8-fluoro-7-methyl-quinoline-5-sulfonyl chloride **96** (50 mg, 0.20 mmol) was
26 dissolved in THF (1.5 mL). To this solution was added DIPEA (106 μL, 0.61 mmol), followed
27 by pyrrolidine (17 μL, 0.20 mmol). The resulting mixture was stirred at room temperature for 15
28 minutes. To the reaction was added potassium *t*-butoxide (610 μL, 0.61 mmol) and stirred at
29 ambient temperature for 1 hour. The reaction mixture was treated with ~2 mL saturated
30 NaHCO₃ and ~2 mL ethyl acetate, agitated vigorously and the layers separated. Solvent was
31 removed from the organic fraction in vacuo to give a brown residue which was purified by
32 automated normal phase chromatography (0-100%EtOAc/hexanes, 10 g silica gel cartridge) to
33 provide 8-*tert*-butoxy-7-methyl-5-pyrrolidin-1-ylsulfonyl-quinoline **97**, 45 mg (63% yield). ¹H
34 NMR (400 MHz, DMSO-*d*₆) δ ppm 8.96 - 9.03 (m, 2 H) 8.06 (s, 1 H) 7.61 - 7.70 (m, 1 H) 3.34
35 (s, 3 H) 3.17 - 3.27 (m, 4 H) 1.72 (dt, *J* = 6.51, 3.44 Hz, 4 H) 1.44 (s, 9 H). MS (ES+) *m/z* 349.0
36 [M+H]⁺.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Step 4. 4 M Hydrogen chloride in dioxane (0.5 mL, 2 mmol) was added to 8-tert-butoxy-
4
5 7-methyl-5-pyrrolidin-1-ylsulfonyl-quinoline **97** (45 mg, 0.12 mmol) and stirred at ambient
6
7 temperature for 2 hours. To this reaction was added Et₂O, collected solid by filtration, washed
8
9 with Et₂O and air-dried to give 7-methyl-5-pyrrolidin-1-ylsulfonyl-quinolin-8-ol hydrochloride
10
11 **30** (35 mg, 92% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.08 (dd, *J* = 8.59, 1.52 Hz, 1 H)
12
13 8.98 (dd, *J* = 4.29, 1.52 Hz, 1 H) 8.06 (d, *J* = 0.51 Hz, 1 H) 7.76 (dd, *J* = 8.84, 4.29 Hz, 1 H)
14
15 3.11 - 3.20 (m, 4 H) 2.43 (s, 3 H) 1.65 - 1.73 (m, 4 H). MS (ES+) *m/z* 293.0 [M+H]⁺.
16
17
18
19
20
21
22

23 **COMT Enzyme Purification**

24
25
26 C-terminal Hexa-His tagged MB-COMT (human and rat) was constructed in a
27
28 pCDNA3.1 vector (Invitrogen, Carlsbad, CA) and expressed in HEK293 cells (ATCC,
29
30 Manassas, VA) using 293 Fectin (Invitrogen, Carlsbad, CA). 300 ml of cells at 83% viability
31
32 were pelleted (15,000 g, 4 °C, 5 minutes) and re-suspended once in PBS and then in membrane
33
34 buffer (15 mM Tris, pH 7.5, 1 mM EGTA, 0.3 mM EDTA, 2 mM MgCl₂, PIC cocktail (Roche,
35
36 Diagnostics, Mannheim Germany)). The samples were then homogenized and frozen and
37
38 defrosted twice in liquid nitrogen. DNase (MilliporeSigma, St. Louis MO) was then added (5 μl
39
40 per ml) and incubated for 10 minutes at room temperature. The samples were then centrifuged at
41
42 40,000 g at 4°C for 25 minutes. The supernatant was discarded, and the pellet was re-suspended
43
44 in Tris-sucrose buffer (20 mM Tris, pH 7.4, 250 mM sucrose) and homogenized again. The
45
46 resulting solution was aliquoted and stored at -80 °C until use. MB-COMT protein concentration
47
48 from the membrane homogenate was estimated using the Bradford Lowry method.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 C-terminal Hexa-His S-COMT (human and rat) was constructed in a pTT5 mammalian
4 cell expression vector (Invitrogen, Carlsbad, CA) and expressed in 293-6E cells (ATTC,
5 Manassas, VA) using linear polyethylenimine (PEI, MilliporeSigma, St. Louis, MO). 0.5 L of
6 cells were harvested at 90% viability and pelleted (15,000 g, 4 C, 5 min). Pellets were frozen at -
7 80 °C. The protein was purified from the pellets using Ni-NTA affinity chromatography and size
8 exclusion per the following: Resin: Ni-NTA (Qiagen, Hilden, Germany) equilibrated in Buffer
9 A. Bed volume: 4 mL. Column: 1.0 cm diameter Econo-column (BioRad, Hercules, CA) Buffer
10 A: 50 mM Tris HCl, 150 mM NaCl, 30 mM imidazole, 2 mM MgCl₂, 0.1 mM TCEP, pH 8.0 (4
11 °C) Buffer B: 50 mM Tris HCl, 150 mM NaCl, 300 mM imidazole, 2 mM MgCl₂, 0.1 mM
12 TCEP, pH 8.0 (4 °C). The target protein in the soluble lysate fraction was batch bound to 4 mL
13 of Ni-NTA resin at 4 °C for 2 hours. The resin was collected in a 1.0 cm diameter Econo-
14 column, was washed with 20 CV of buffer A, and the protein of interest was eluted in Buffer B.
15 Fractions (1 mL) were collected. The final destination buffer was 100 mM potassium phosphate,
16 1mM DTT, 5 mM MgCl₂, 20% glycerol at pH 7.2.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

Compound dilution

36
37
38

39 All compounds were diluted to a final concentration of 1.5% DMSO from 10 mM stocks
40 in two steps. Stock (7.5 µl) was diluted serially into 15 µl of DMSO. Then 4.5 µl of the previous
41 was diluted into 55.5 µl of COMT assay buffer (50 mM Tris, 5-10 mM MgCl₂, 2.5 mM DTT, pH
42 6.9). 1 µl of that was then deposited into a Corning® low volume 384 well white flat bottom
43 polystyrene NBS™ microplate in triplicate.
44
45
46
47
48
49
50

51 For 0 and 100% inhibition controls, DMSO and Tolcapone (synthesized in house) were
52 added to each assay plate. DMSO was added to 12 wells on the plate in a final concentration of
53 1.5% and Tolcapone was added to 12 wells on the plate with a final concentration of 10 µM.
54
55
56
57
58
59
60

1
2
3 Tolcapone was diluted to 666.6 μM and then diluted in two steps according to the
4
5 procedure of the compounds above. DMSO (MilliporeSigma, St. Louis, MO) was also diluted
6
7 according to the same scheme.
8
9

10 **Enzyme reaction**

11
12
13 COMT activity was measured using the MTase Glo Methyltransferase Assay (Promega,
14
15 Madison, WI) according to manufacturer's instructions. Assays were carried out in Corning®
16
17 low volume 384 well white flat bottom polystyrene NBS™ microplates with a final volume of
18
19 5 μL containing approximately 7 ng of human MB-COMT as estimated by the Bradford Lowry
20
21 method from the membrane homogenate, 4 ng of rat MB-COMT as estimated by the Bradford
22
23 Lowry method from the membrane homogenate, 1 ng of Human S-COMT or 1 ng of Rat S-
24
25 COMT respectively. All reactions contained 20 μM high purity S-adenosyl methionine (SAM,
26
27 CisBio, Bedford, MA) in COMT assay buffer (50 mM Tris, 5-10 mM MgCl_2 , 2.5 mM DTT, pH
28
29 6.9). For MB-COMT, the catechol substrate was 7 μM norepinephrine (MilliporeSigma, St.
30
31 Louis, MO) and for S-COMT the substrate was 10 μM 7,8-dihydroxy-4-methylcoumarin³²
32
33 (MilliporeSigma, St. Louis, MO).
34
35
36
37
38

39
40 Reactions were performed in a 37°C incubator for 1 hr. The plate was removed from the
41
42 incubator and allowed to cool to room temperature for 15min. MTase reagent A (Promega,
43
44 Madison, WI) was first diluted 1 to 5 into RO water and 1 μl was then added to the well. The
45
46 plate was spun down, shaken and allowed to incubate for 30 minutes at room temperature
47
48 avoiding light. Then 5 μl of MTase reagent B (Promega, Madison, WI) were added to all of the
49
50 wells. The plate was spun down, shaken and allowed to incubate for 30 minutes at room
51
52 temperature avoiding light. Luminescence was detected with a Tecan Infinite M100 Pro plate
53
54 reader.
55
56
57
58
59
60

Standard curve

A standard curve was run on every plate. The amount of S-adenosyl homocysteine (SAH, CisBio, Bedford, MA) produced was determined using a standard curve and a linear back-calculation method. The standard curve comprised of varying concentrations of SAH from 500 nM down to 0 nM while maintaining a final SAM/SAH concentration of 50 μ M. In order to correct for background levels present in the enzymatic lysate (MB-COMT), enzyme at assay concentration was added to the standard curve as well.

Determination of Inhibition

Percentage inhibition was calculated by using 10 μ M Tolcapone value as 100% inhibition value and the DMSO control as the 0% inhibition value. The dose response curves were constrained at 0% inhibition while keeping the percentage inhibition of the highest compound concentration floating. IC_{50} was determined by non-linear regressions and curve fitting using a 4-parameter fit with a variable slope in the Dotmatics® studies program.

Potency data presented is an average of three separate experiments in which each data point was run in triplicate (unless otherwise noted) and reported as $pIC_{50} \pm SEM$ in Tables 1 and 2.

X-Ray Crystallography

Following the protocol from M. Ellermann et al.³³ 1 μ M rat S-COMT was incubated overnight at 4 °C with 10 μ M compound **21** and 1 mM SAM (Sigma-Aldrich) in 50 mM Tris-HCl pH 7.6, 50 mM NaCl, 10 mM DTT, 2 mM $MgCl_2$, concentrated to 13 mg mL⁻¹ and cleared by centrifugation. Crystallization was set up at 18 °C with hanging drop vapour diffusion and

1
2
3 crystals grew in 100 mM Bis-Tris propane pH 5.5-7.0, 100 mM NaCl, 1.2-1.8 M (NH₄)₂SO₄. The
4
5 crystal was cryo-protected in 25 mM Bis-Tris propane pH 7.3, 40% (w/v) PEG3350, 150 mM
6
7 sodium formate and flash-cooled in liquid nitrogen for data collection. Diffraction data were
8
9 collected at 100 K using a MicroMax-007 HF rotating anode X-ray generator (Rigaku) and
10
11 mar345 image plate detector (marXperts). Data were integrated and scaled using MOSFLM³⁴
12
13 and SCALA³⁵. The structure was determined by molecular replacement using PHASER³⁶ (CCP4
14
15 program suite³⁷) and the published S-COMT structure (PDB accession code: 3S68)³³, without
16
17 ligand, as search model. Structural refinement was performed with PHENIX³⁸ with altering
18
19 cycles of manual model building with COOT³⁹. The crystallographic statistics are summarized in
20
21 Supplementary Table S3. The protein structure was validated using MolProbity⁴⁰. The
22
23 coordinates and structure factors have been deposited in the Protein Data Bank (entry code
24
25 6GY1). Molecular pictures are prepared using program PyMol⁴¹.
26
27
28
29
30
31

32 **Mouse Blood-Brain Barrier (BBB) Assay**

33 *Animals*

34
35
36
37
38
39
40 Male, C57BL/6J mice (8 weeks old) were purchased from The Jackson Laboratory (Bar Harbor,
41
42 ME, USA). The mice were group-housed (5/cage) and allowed to habituate to the colony room
43
44 for one week before handling. All mice were handled on alternating days (3X/week) during the
45
46 following week and again on the day before testing to acclimate the mice to the experimenter.
47
48

49 All procedures were approved by the Sobran, Inc. Rangos Facility Animal Care and Use
50
51 Committee and followed the *Guide for the Care and Use of Laboratory Animals*.
52
53

54 *Drugs*

1
2
3 Novel COMT inhibitors (synthesized in-house) were suspended in vehicle (0.1% Tween80, 0.1%
4 1510 silicone antifoam, 1% methylcellulose 400c/p in water) and administered through
5
6 intraperitoneal (ip) injection. The administration volume was 10 mL/kg.
7
8
9

10 11 *BBB Protocol*

12
13
14
15 On the day of testing, mice were moved from the colony room to a holding room where they
16 were weighed. Drug injections were initiated after an hour acclimation period. Each mouse
17 received a single injection of the test compound (10 mg/kg). At the appropriate time point (5, 15,
18 or 60 minutes post injection), mice were moved individually to the procedure table where they
19 were anaesthetized via isoflurane. The chest cavity was opened and blood was collected via
20 cardiac puncture into Lithium-Heparin 1.3mL microtubes (Sarstadt, Numbrecht, Germany). The
21 mouse was then perfused with phosphate buffered saline (PBS) via a 12mL syringe using a 23
22 gauge needle to clear the brain of any residual blood and the brain was harvested. Blood was
23 then centrifuged at 2000 rpm at 4°C for 15 minutes to separate the plasma. Plasma was then
24 transferred into Thermo Scientific Matrix tubes for storage. Brains were collected in 5mL
25 Thermo Scientific Nunc tubes and homogenized in a 4x (w/v) volume of PBS, pH 7.4. Plasma
26 and brain homogenate samples were stored at -80°C until analysis.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48

49 **Rat Biomarker Assay**

50 51 52 *Animals*

1
2
3 Male Sprague Dawley rats (176-200 g on arrival; Charles River Laboratories, Wilmington, MA,
4 USA) were used for all studies. Rats were allowed to habituate to the colony room for one week
5 before handling. All rats were handled individually for at least one minute on each of the three
6 days preceding the day of the experiment. All procedures were approved by the Sobran, Inc.
7 Rangos Facility Animal Care and Use Committee and followed the *Guide for the Care and Use*
8 *of Laboratory Animals*.

18 *Drugs*

20
21
22 Novel COMT inhibitors (synthesized in-house) and tolcapone (synthesized in-house) were
23 suspended in vehicle (0.1% Tween80, 0.1% 1510 silicone antifoam, 1% methylcellulose 400c/p
24 in water) and administered either orally (po) or through intraperitoneal (ip) injection (tolcapone).
25 Administration volumes were 10 mL/kg and 5 mL/kg for po and ip dosing, respectively.

33 *Biomarker protocol*

35
36
37 On the day of testing, rats were transferred to a holding room and weighed. After an hour
38 acclimation period, rats received either vehicle or a COMT inhibitor. At the 4 hour time point,
39 rats were moved to a separate procedure room where they were anaesthetized via isoflurane.
40
41 Once the rats were determined to be unresponsive, fur on their heads was shaved using electric
42 clippers. The rats were positioned in a stereotaxic frame, with their heads pointed down at a 45-
43 degree angle. To collect cerebrospinal fluid (CSF), previously published protocols⁴² were
44 adapted. Briefly, we used a 23G needle connected via PE50 tubing to a collection syringe to
45 access the cisterna magna according to the protocol. Slight negative pressure was used to ensure
46 the CSF flowed evenly. During collection, we observed the CSF as it flowed through the tubing
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 into the syringe looking for discoloration due to blood contamination. If any blood was observed
4 entering the tubing, a cut was made in the tubing above the blood to minimize the contamination
5 of the CSF sample and only the CSF above the cut was further processed. The CSF was then
6 transferred to previously chilled (dry ice) Eppendorf tubes containing 0.05M perchloric acid (4:1
7 CSF:perchloric acid ratio). The tubes were put back on dry ice until the end of the procedure.
8
9 Any samples with visible blood contamination (pink/red color) were not analyzed. Next, the
10 chest cavity was opened, and blood was collected by cardiac puncture. The blood was collected
11 in Lithium-Heparin 1.3mL microtubes (Sarstadt, Numbrecht, Germany) and stored on ice. The
12 rat was then transcardially perfused with ice-cold phosphate buffered saline (PBS) via a
13 peristaltic pump set to a flow rate of 20 mL/min to clear the brain of any residual blood and the
14 brains were collected in 5mL Thermo Scientific Nunc tubes. Blood was then centrifuged at 2000
15 rpm at 4°C for 15 minutes to separate the plasma. Plasma was then transferred into Thermo
16 Scientific Matrix tubes for storage. CSF, plasma, and brains were stored at -80°C until analysis.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 *Bioanalysis protocol*

35
36
37
38 Artificial CSF (aCSF) consisted of 125mM NaCl, 5mM potassium chloride, 1mM MgCl₂, 5mM
39 D-glucose, 1.25mM sodium phosphate di-basic, 2mM calcium chloride and 24mM NaHCO₃.
40
41 Triplicate standards and QCs of HVA and DOPAC were prepared in 0.01M perchloric acid in
42 aCSF. Triplicate 10μL standards, QCs, and CSF samples were transferred to a 96-well Corning
43 Axygen ® PCR plate (Corning, NY). 10μL of 100mM sodium tetraborate (MilliporeSigma, St.
44 Louis, MO) in water and 2μL of internal standard consisting of 500 ng/mL each of HVA-d5 and
45 DOPAC-d5 (both C/D/N Isotopes Pointe-Claire, Quebec, CA) in 50% methanol in water (v/v)
46 were added to each sample and the plate was mix for 2 minutes at 1000 rpm. To this was added
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 10 μ L of 2% benzoyl chloride in acetonitrile (v/v) to each sample, and the plate was mixed for 4
4 minutes at 1000 rpm. From the mixture, 5 μ L of each sample was injected for analysis using an
5 Agilent 6540 QTOF with Jet Stream Electrospray Ionization Source (ESI) and Agilent 1290
6 UHPLC. Solvents were 10mM ammonium formate in water (A) and 100% acetonitrile (B).
7
8 Chromatographic separation was achieved over 7.5 minutes using a Phenomenex Luna Omega
9
10 2.1 x 100mm, 1.6 μ m, C18 column with a binary gradient starting 21% B. The flow rate was
11
12 500 μ l min⁻¹. The autosampler was set at 20 °C. The LC gradient was as follows: 0–4 min, 21%
13
14 B; 4 – 4.5 min, 21-40% B; 4.5 – 5.0min, 40% B. 5.01 – 6.0 min, 95%B, 6.01 min, 21% B. The
15
16 mass spec acquisition was performed using full scan MS from m/z 50 to 800 with the following
17
18 source conditions: Drying and Sheath Gas temperatures at 350°C and 400°C, respectively; Both
19
20 gas flows at 12L/min; Nebulizer- 45psig, VCap, Nozzle and Fragmenter voltages at 3000V,
21
22 600V and 100V, respectively. The ammonium adducts of the dual benzoylated DOPAC and
23
24 DOPAC-d5 (m/z 394.1285 and 399.1599, respectively) and the single benzoylated HVA and
25
26 HVA-d5 (m/z 304.1179 and 309.1493, respectively) were used for data analysis. Analyte peak
27
28 areas were determined from the extracted ion chromatograms within a +/- 20ppm window.
29
30 Concentrations were calculated with linear regression analysis using Agilent Masshunter
31
32 Quantitative Analysis Software (B.06.00 SP01).
33
34
35
36
37
38
39
40
41
42
43

44 **ASSOCIATED CONTENT**

45 **Supporting Information**

46
47 (Tables of raw data for mouse and rat brain penetration, details for the preparation of Rat S-
48
49 COMT and the crystallographic details, Molecular formula strings (CSV))
50
51

52 **Accession Codes**

The PDB accession code for the X-ray structure of **21** bound to Rat S-COMT is 6GY1. Authors will release the atomic coordinates and experimental data upon article publication.

AUTHOR INFORMATION

Corresponding Author

* Phone +1 410 955 0894 Fax: +1 410 955 1044 Email james.barrow@libd.org

ORCID

James C Barrow 0000-0001-5115-9300

Author Contributions

Designed and synthesized compounds (IB, PdL, GE, YH, MP, DS, FM, JCB)

Developed the in vitro assays (MK, HW, MW, EJ, JCB)

Developed the in vivo assays (DA, VA, GC, MD, AK, EJ, DS, JCB)

Crystallization and structure refinement (MSS)

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge David Boucaut (UCB) for assistance with in vivo studies and Eric Gillent (UCB) and Benoit Culot (UCB) for bioanalysis assistance.

1
2
3 Funding of parts of this work from the National Institutes of Mental Health NIMH R01
4
5 MH107126 is gratefully acknowledged.
6
7
8
9

10 **ABBREVIATIONS USED**

11
12 %F, bioavailability; ACN, acetonitrile; aCSF, artificial cerebrospinal fluid; ADHD, attention
13
14 deficit hyperactivity disorder; ADME, absorption, distribution, metabolism, and excretion;
15
16 AlCl₃, aluminum chloride; BCRP, breast cancer resistance protein; Bis-Tris, Bis(2-
17
18 hydroxyethyl)aminotris(hydroxymethyl)methane; BLQ, below limit of quantitation; BnBr,
19
20 benzylbromide; BnOH, benzyl alcohol; CHCl₃, chloroform; CL_p, plasma clearance; COMT,
21
22 catechol *O*-methyltransferase; Cs₂CO₃, cesium carbonate; CSF, cerebrospinal fluid; CsF, cesium
23
24 fluoride; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMDN, N,N-
25
26 dimethylethylenediamine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DNase,
27
28 deoxyribonuclease; DOPAC, dihydroxyphenyl acetic acid; DTT, dithiothreitol; EDTA,
29
30 ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-
31
32 tetraacetic acid; ER, efflux ratio; ESI, electrospray ionization; Et, ethyl; EtOAc, ethyl acetate;
33
34 HBr, hydrobromic acid; HCl, hydrochloric acid; HEK, human embryonic kidney cells 293;
35
36 HPLC, high-performance liquid chromatography; HVA, homovanillic acid; ip, intraperitoneal;
37
38 iv, intravenous; K₂CO₃, potassium carbonate; K_{p u,u}, concentration ratio of unbound drug in brain
39
40 to unbound drug in plasma; LC, liquid chromatography; LC-MS, liquid chromatography–mass
41
42 spectrometry; L-DOPA, L-3,4-dihydroxyphenylalanine; M, molar; m, mouse; MB-COMT,
43
44 membrane bound catechol *O*-methyltransferase; mCPBA, meta-chloroperoxybenzoic acid;
45
46 MDCK, Madin-Darby canine kidney cells; MeOH, methanol; MgCl₂, magnesium chloride;
47
48 MgSO₄, magnesium sulfate; mpk, milligram per kilogram; MRP2, multidrug resistance-
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 associated protein 2; MTase, methyltransferase; N, normal; N/A, not applicable; NaCl, sodium
4 chloride; NaH, sodium hydride; NaHCO₃, sodium bicarbonate; NaOH, sodium hydroxide; NBS,
5
6 N-bromosuccinimide; NCS, N-chlorosuccinimide; Ni-NTA, nickel-nitrilotriacetic acid; NIS, N-
7
8 iodosuccinimide; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; PCR,
9
10 polymerase chain reaction; Pd(PPh₃)₄, Tetrakis(triphenylphosphine)palladium(0); Pd₂(dba)₃,
11
12 tris(dibenzylideneacetone)-dipalladium(0); PDB, Protein Data Bank; PE, polyethylene; PEG,
13
14 polyethylene glycol; PFC, prefrontal cortex; P-gp, P-glycoprotein 1; Ph, phenyl; PIC cocktail,
15
16 protease inhibitor cocktail; pIC₅₀, -log(IC₅₀) where IC₅₀ represents the compound/substance
17
18 concentration required for 50% inhibition; PK, pharmacokinetic; PMB, *p*-methoxybenzyl; PMB-
19
20 Cl, *p*-methoxybenzyl chloride; po, oral; ppm, parts per million; QC, quality control standard;
21
22 QTOF, quadrapole time of flight; r, rat; RO, reverse osmosis; rpm, revolutions per minute; SAH,
23
24 S-adenosyl-L-homocysteine; SAM, S-adenosyl methionine; SAR, structure–activity relationship;
25
26 S-COMT, soluble catechol *O*-methyltransferase; SnAR, nucleophilic aromatic substitution;
27
28 T1/2, half-life of the product; TBAF, tetrabutylammonium fluoride; TCEP, (tris(2-
29
30 carboxyethyl)phosphine); TFA, trifluoroacetic acid; THF, tetrahydrofuran; TM, trademark;
31
32 TMS, tetramethylsilane; TMSE, 2-(trimethylsilyl)ethyl;
33
34 Tris, tris(hydroxymethyl)aminomethane; Tris-HCl, tris(hydroxymethyl)aminomethane
35
36 hydrochloride; UHPLC, ultra high performance liquid chromatography; UV, ultraviolet; V_{ss},
37
38 volume of distribution; Xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene
39
40
41
42
43
44
45
46
47
48

49 REFERENCES

- 50
51 1. Lachman, H. M.; Papolos, D. F.; Saito, T.; Yu, Y. M.; Szumlanski, C. L.; Weinshilboum,
52
53 R. M., Human catechol-*O*-methyltransferase pharmacogenetics: Description of a functional
54
55
56
57
58
59
60

1
2
3 polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics*
4
5 **1996**, *6*, 243-250.

6
7
8 2. (a) Kaenmaki, M.; Tammimaki, A.; Myohanen, T.; Pakarinen, K.; Amberg, C.;
9
10 Karayiorgou, M.; Gogos, J. A.; Mannisto, P. T., Quantitative role of COMT in dopamine
11
12 clearance in the prefrontal cortex of freely moving mice. *J Neurochem* **2010**, *114*, 1745-1755; (b)
13
14 Yavich, L.; Forsberg, M. M.; Karayiorgou, M.; Gogos, J. A.; Mannisto, P. T., Site-specific role
15
16 of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal
17
18 striatum. *J Neurosci* **2007**, *27*, 10196-10202.

19
20
21 3. Mannisto, P. T.; Kaakkola, S., Catechol-O-methyltransferase (COMT): Biochemistry,
22
23 molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors.
24
25 *Pharmacol Rev* **1999**, *51*, 593-628.

26
27
28 4. Lotta, T.; Vidgren, J.; Tilgmann, C.; Ulmanen, I.; Melen, K.; Julkunen, I.; Taskinen, J.,
29
30 Kinetics of human soluble and membrane-bound catechol O-methyltransferase - a revised
31
32 mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* **1995**, *34*,
33
34 4202-4210.

35
36
37 5. Papaleo, F.; Crawley, J. N.; Song, J.; Lipska, B. K.; Pickel, J.; Weinberger, D. R.; Chen,
38
39 J., Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress
40
41 reactivity in mice. *J Neurosci.* **2008**, *28*, 8709-8723.

42
43
44 6. Reenila, I.; Mannisto, P. T., Catecholamine metabolism in the brain by membrane-bound
45
46 and soluble catechol-O-methyltransferase (COMT) estimated by enzyme kinetic values. *Medical*
47
48 *Hypotheses* **2001**, *57*, 628-632.

49
50
51 7. Learmonth, D. A.; Kiss, L. E.; Soares-da Silva, P., The chemistry of catechol O-
52
53 methyltransferase inhibitors. *Int Rev Neurobiol* **2010**, *95*, 119-162.

- 1
2
3 8. Bonifacio, M. J.; Palma, P. N.; Almeida, L.; Soares-Da-Silva, P., Catechol-O-
4 methyltransferase and its inhibitors in Parkinson's disease. *CNS Drug Reviews* **2007**, *13*, 352-
5
6 379.
7
- 8
9
10 9. Russ, H.; Müller, T.; Woitalla, D.; Rahbar, A.; Hahn, J.; Kuhn, W., Detection of
11
12 tolcapone in the cerebrospinal fluid of parkinsonian subjects. *Naunyn-Schmiedeberg's Archives*
13
14 *of Pharmacology* **1999**, *360* (6), 719-720.
15
- 16
17 10. Müller, T., Tolcapone addition improves Parkinson's disease associated nonmotor
18
19 symptoms. *Ther Adv Neurol Diso* **2014**, *7*, 77-82.
20
- 21
22 11. Tunbridge, E. M.; Harrison, P. J.; Weinberger, D. R., Catechol-O-methyltransferase,
23
24 cognition, and psychosis: Val(158)Met and beyond. *Biol Psychiat* **2006**, *60*, 141-151.
25
- 26
27 12. Olanow, C. W.; Watkins, P. B., Tolcapone. *Clinical Neuropharmacology* **2007**, *30*, 287-
28
29 294.
30
- 31
32 13. Axelrod, J., O-Methylation of epinephrine and other catechols in vitro and in vivo.
33
34 *Science* **1957**, *126*, 400-401.
35
- 36
37 14. Ross, S. B.; Haljasmaa, O., Catechol-O-methyl transferase inhibitors - in vitro inhibition
38
39 of enzyme in mouse brain extract. *Acta Pharmacol Tox* **1964**, *21*, 205-214.
40
- 41
42 15. Borchardt, R. T.; Thakker, D. R.; Warner, V. D.; Mirth, D. B.; Sane, J. N., Catechol O-
43
44 methyltransferase .8. Structure-activity-relationships for inhibition by 8-hydroxyquinolines. *J*
45
46 *Med Chem* **1976**, *19*, 558-560.
47
- 48
49 16. Barrow, J. C., Inhibitors of catechol-O-methyltransferase. *CNS & Neurological*
50
51 *Disorders-Drug Targets* **2012**, *11*, 324-332.
52
- 53
54 17. Almeida, L.; Rocha, J. F.; Falca, A.; Palma, P. N.; Loureiro, A. I.; Pinto, R.; Bonifacio,
55
56 M. J.; Wright, L. C.; Nunes, T.; Soares-da-Silva, P., Pharmacokinetics, pharmacodynamics and
57
58
59
60

1
2
3 tolerability of opicapone, a novel catechol-O-methyltransferase inhibitor, in healthy subjects.

4
5 *Clin Pharmacokinet* **2013**, *52*, 139-151.

6
7
8 18. Barrow, J., Ernst, G.; Huang, Y.; Buchler, I.; Weinberger, D. COMT Inhibiting Methods
9 and Compositions. WO2016123576, 2016.

10
11
12 19. Liu, X. R.; Chen, C. P., Free drug hypothesis for CNS drug candidates. In *Blood-Brain*
13 *Barrier in Drug Discovery*: Di, L.; Kerns, E.H., Eds. Wiley, Hoboken, New Jersey, **2015**, 42-65.

14
15
16 20. Hutson, P. H.; Curzon, G., Dopamine metabolites in rat cisternal cerebrospinal-fluid -
17 major contribution from extrastriatal dopamine neurons. *J Neurochem* **1986**, *46*, 186-190.

18
19
20 21. Robinson, R. G.; Smith, S. M.; Wolkenberg, S. E.; Kandebo, M.; Yao, L. H.; Gibson, C.
21 R.; Harrison, S. T.; Polsky-Fisher, S.; Barrow, J. C.; Manley, P. J.; Mulhearn, J. J.; Nanda, K. K.;
22 Schubert, J. W.; Trotter, B. W.; Zhao, Z. J.; Sanders, J. M.; Smith, R. F.; McLoughlin, D.;
23 Sharma, S.; Hall, D. L.; Walker, T. L.; Kershner, J. L.; Bhandari, N.; Hutson, P. H.; Sachs, N. A.,
24 Characterization of non-nitrocatechol pan and isoform specific catechol-O-methyltransferase
25 inhibitors and substrates. *ACS Chem Neurosci* **2012**, *3*, 129-140.

26
27
28 22. Lin, J. H., CSF as a surrogate for assessing CNS exposure: An industrial perspective.
29 *Curr Drug Metab* **2008**, *9*, 46-59.

30
31
32 23. (a) Banks, W. A., From blood-brain barrier to blood-brain interface: new opportunities
33 for CNS drug delivery. *Nat Rev Drug Discov* **2016**, *15* (4), 275-292; (b) Rankovic, Z., CNS drug
34 design: Balancing physicochemical properties for optimal brain exposure. *J Med Chem* **2015**, *58*,
35 2584-2608.

36
37
38 24. Muller, N.; Lopicque, F.; Monot, C.; Payan, E.; Gillet, P.; Bannwarth, B.; Netter, P.,
39 Protein-binding of indomethacin in human cerebrospinal-fluid. *Biochem Pharmacol* **1991**, *42*,
40 799-804.

- 1
2
3 25. Ganrot, K.; Laurell, C. B., Measurement of IgG and albumin content of cerebrospinal-
4 fluid, and its interpretation. *Clin Chem* **1974**, *20*, 571-573.
5
6
7 26. Vidgren, J.; Svensson, L. A.; Liljas, A., Crystal-structure of catechol O-
8 methyltransferase. *Nature* **1994**, *368*, 354-358.
9
10
11 27. Lundstrom, K.; Tenhunen, J.; Tilgmann, C.; Karhunen, T.; Panula, P.; Ulmanen, I.,
12 Cloning, expression and structure of catechol-O-methyltransferase. *Biochimica Et Biophysica*
13 *Acta-Protein Structure and Molecular Enzymology* **1995**, *1251*, 1-10.
14
15
16 28. (a) Bai, H. W.; Shim, J. Y.; Yu, J.; Zhu, B. T., Biochemical and molecular modeling
17 studies of the O-methylation of various endogenous and exogenous catechol substrates catalyzed
18 by recombinant human soluble and membrane-bound catechol-O-methyltransferases. *Chem Res*
19 *Toxicol* **2007**, *20*, 1409-1425; (b) Magarkar, A.; Parkkila, P.; Viitala, T.; Lajunen, T.; Mobarak,
20 E.; Licari, G.; Cramariuc, O.; Vauthey, E.; Rog, T.; Bunker, A., Membrane bound COMT
21 isoform is an interfacial enzyme: general mechanism and new drug design paradigm. *Chem*
22 *Commun* **2018**, *54*, 3440-3443.
23
24
25 29. (a) Suzuki, H.; Abe, H., Copper-assisted displacement reaction of nonactivated
26 iodoarenes with arenesulfinates - convenient alternative synthesis of unsymmetrical diaryl
27 sulfones. *Tetrahedron Lett* **1995**, *36*, 6239-6242; (b) Zhu, W.; Ma, D. W., Synthesis of aryl
28 sulfones via L-proline-promoted CuI-catalyzed coupling reaction of aryl halides with sulfinic
29 acid salts. *J Org Chem* **2005**, *70*, 2696-2700.
30
31
32 30. (a) Mispelaere-Canivet, C.; Spindler, J. F.; Perrio, S.; Beslin, P., Pd-2(dba)(3)/Xantphos-
33 catalyzed cross-coupling of thiols and aryl bromides/triflates. *Tetrahedron* **2005**, *61*, 5253-5259;
34
35 (b) Mase, T.; Itoh, T., General and practical synthesis of benzothiazoles. *Pure Appl Chem* **2008**,
36 *80*, 707-715.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 31. Manske, R. H. F.; Kulka, M., The Skraup synthesis of quinolines. *Org Reactions* **1953**, *7*,
4 59-98.
5
6
7
8 32. Qian, X. K.; Wang, P.; Xia, Y. L.; Dou, T. Y.; Jin, Q.; Wang, D. D.; Hao, D. C.; Bi, X.
9 L.; Ge, G. B.; Yang, L., A highly selective fluorescent probe for sensing activities of catechol-O-
10 methyltransferase in complex biological samples. *Sensor Actuat B-Chem* **2016**, *231*, 615-623.
11
12
13
14 33. Ellermann, M.; Lerner, C.; Burgy, G.; Ehler, A.; Bissantz, C.; Jakob-Roetne, R.; Paulini,
15 R.; Allemann, O.; Tissot, H.; Grunstein, D.; Stihle, M.; Diederich, F.; Rudolph, M. G., Catechol-
16 O-methyltransferase in complex with substituted 3'-deoxyribose bisubstrate inhibitors. *Acta*
17 *Crystallogr D Biol Crystallogr* **2012**, *68*, 253-260.
18
19
20
21
22 34. Batty, T. G.; Kontogiannis, L.; Johnson, O.; Powell, H. R.; Leslie, A. G., iMOSFLM: a
23 new graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr D*
24 *Biol Crystallogr* **2011**, *67*, 271-281.
25
26
27
28
29 35. Evans, P., Scaling and assessment of data quality. *Acta Crystallogr D Biol Crystallogr*
30 **2006**, *62*, 72-82.
31
32
33
34 36. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.;
35 Read, R. J., Phaser crystallographic software. *J Appl Crystallogr* **2007**, *40*, 658-674.
36
37
38
39 37. Murshudov, G. N.; Skubak, P.; Lebedev, A. A.; Pannu, N. S.; Steiner, R. A.; Nicholls, R.
40 A.; Winn, M. D.; Long, F.; Vagin, A. A., REFMAC5 for the refinement of macromolecular
41 crystal structures. *Acta Crystallogr D Biol Crystallogr* **2011**, *67*, 355-367.
42
43
44
45 38. Collaborative Computational Project, N., The CCP4 suite: programs for protein
46 crystallography. *Acta Crystallogr D Biol Crystallogr* **1994**, *50*, 760-763.
47
48
49
50 39. Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K., Features and development of Coot.
51 *Acta Crystallogr D Biol Crystallogr* **2010**, *66*, 486-501.
52
53
54
55
56
57
58
59
60

- 1
2
3 40. Chen, V. B.; Arendall, W. B., 3rd; Headd, J. J.; Keedy, D. A.; Immormino, R. M.;
4
5 Kapral, G. J.; Murray, L. W.; Richardson, J. S.; Richardson, D. C., *MolProbity: all-atom*
6
7 *structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr*
8
9 **2010**, *66*, 12-21.
- 10
11
12 41. DeLano, W., *The PyMOL molecular graphics system. Version 12r3pre, Schroedinger,*
13
14 *LLC 2002.*
- 15
16
17 42. (a) Nirogi, R.; Kandikere, V.; Mudigonda, K.; Bhyrapuneni, G.; Muddana, N.; Saralaya,
18
19 R.; Benade, V., *A simple and rapid method to collect the cerebrospinal fluid of rats and its*
20
21 *application for the assessment of drug penetration into the central nervous system. J Neurosci*
22
23 *Meth 2009*, *178*, 116-119; (b) Mahat, M. Y. A.; Ahamed, N. F. A.; Chandrasekaran, S.;
24
25 Rajagopal, S.; Narayanan, S.; Surendran, N., *An improved method of transcutaneous cisterna*
26
27 *magna puncture for cerebrospinal fluid sampling in rats. J Neurosci Meth 2012*, *211*, 272-279.

30
31
32
33
34
35 Graphical Abstract:

