Journal of Medicinal Chemistry

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Drug Annotation

Discovery of 4-((2*S*,4*S*)-4-Ethoxy-1-((5-methoxy-7-methyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid (LNP023), a Factor B Inhibitor Specifically Designed To Be Applicable to Treating a Diverse Array of Complement Mediated Diseases

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Cite This: https://dx.doi.org/10.1021/acs.jmedchem.9b01870

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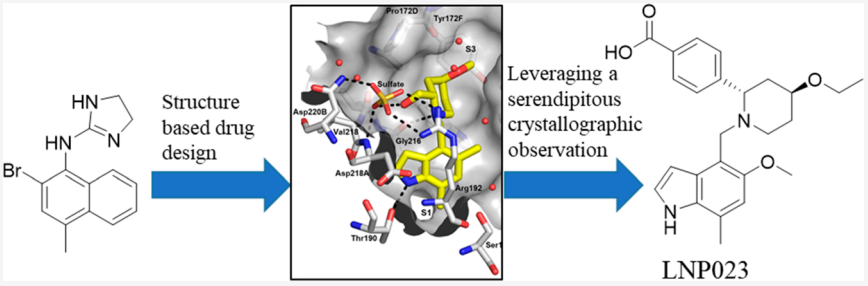

ABSTRACT: The alternative pathway (AP) of the complement system is a key contributor to the pathogenesis of several human diseases including age-related macular degeneration, paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and various glomerular diseases. The serine protease factor B (FB) is a key node in the AP and is integral to the formation of C3 and C5 convertase. Despite the prominent role of FB in the AP, selective orally bioavailable inhibitors, beyond our own efforts, have not been reported previously. Herein we describe in more detail our efforts to identify FB inhibitors by high-throughput screening (HTS) and leveraging insights from several X-ray cocrystal structures during optimization efforts. This work culminated in the discovery of LNP023 (**41**), which is currently being evaluated clinically in several diverse AP mediated indications.

INTRODUCTION

The complement system is a major means of eliminating foreign pathogens and is a critical component of the innate immune system. The role of complement in defending against foreign pathogens has been known for over 100 years.[1] Complement is now recognized as a contributing factor to a wide range of human diseases of noninfectious origin as well.[2] There are three pathways that comprise the complement system, the classical, lectin, and alternative pathways, all of which converge on a key nodal protein, C3. In addition, the alternative pathway (AP) serves as an amplification loop of complement activation. The alternative pathway, in particular, has come to prominence as a contributor to various human diseases[3] including age-related macular degeneration (AMD),[4,5] paroxysmal nocturnal hemoglobinuria (PNH),[6] atypical hemolytic uremic syndrome (aHUS),[7] and various glomerular diseases (e.g., C3 glomerulopathy (C3G)).[8] Components of the complement system that are limited to the AP include the proteases factor D (FD) and factor B (FB), as well as the γ globulin protein properdin (factor P). Due to

Received: November 11, 2019

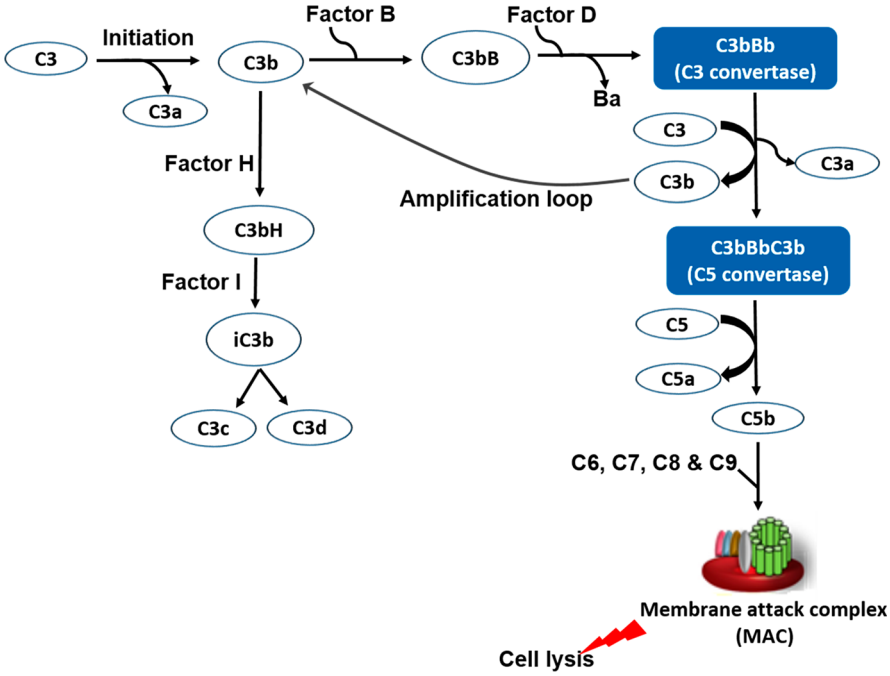

Figure 1. Schematic representation of the alternative complement pathway (AP) from initiation to cell lysis, as well as representing the breakdown of C3b via the actions of factor I and factor H.

strong association of the alternative pathway with many diseases of high unmet need, we began drug discovery programs against all three targets. Our efforts toward low molecular weight inhibitors of FD have been described previously,[9−11] as has our development of an antibody targeting factor P.[12] We recently disclosed our efforts to target FB as well.[13] Herein we describe in more detail our efforts to develop low molecular weight inhibitors of FB culminating in the discovery of LNP023 which is currently being evaluated clinically in several different indications.

FB is a trypsin-like serine protease (93 kDa) that circulates in human blood in a latent form at a relatively high concentration of approximately 3 μM.[14−16] FB can bind to C3b or C3(H_2O) to generate pro-C3 convertase (C3bB) causing a conformational change that enables the complex to become a substrate for FD. FD cleaves the Arg234−Lys235 bond of FB in this complex, liberating the N-terminal fragment (Ba), while the remaining FB subunit (Bb), which contains the serine protease domain, becomes a critical component of the now fully activated C3 convertase.[17−19] Of note, C3 convertase can cleave additional C3 to produce more C3b, thereby further amplifying the local complement response. Binding of a second C3b molecule to the C3 convertase complex in the presence of excess C3b generates a C5 convertase complex, C3bBbC3b, thereby shifting substrate specificity from C3 to C5 and leading to membrane attack complex (MAC) formation, which results in cell lysis and release of C5a, a major chemotaxin (Figure 1).

RESULTS AND DISCUSSION

At the outset of our program we strived for a compound profile that was suitable for oral dosing and would therefore enable treatment of an array of AP mediated diseases impacting a diverse set of target tissues, e.g., PNH (red blood cells), C3G (kidney), and AMD (eye). The desire to design inhibitors as an AMD therapy posed an additional hurdle. While FB is primarily synthesized by the liver, it is also known that FB can be locally produced in the eye by retinal pigment epithelial (RPE) cells.[20] There are conflicting reports whether the fraction of FB present in the RPE layer of the eye is locally produced or systemically derived,[20,21] so we aimed for a molecule that could inhibit FB mediated complement activation both in the blood and also locally in the posterior tissues of the eye. Targeting the posterior tissues of the eye (e.g., the retina and RPE/choroid) poses additional drug discovery challenges; for example, the neural retina, being part of the central nervous system, is protected by the blood−retinal barrier (BRB). The BRB serves a similar function as the blood−brain barrier and can be a formidable obstacle.[22]

Recognizing the aforementioned challenges, it was clear that the known covalent inhibitors of FB, including pan protease inhibitors nafamostat (FUT-175),[23] sepimostat (FUT-187)[24] as well as various peptide aldehydes,[25] lack the specificity and/or pharmacokinetic profiles suitable for our needs. In addition, it was recognized that some of the unique structural aspects of FB, in particular in the serine protease domain (SPD), may require a different targeting strategy than we have employed with success for other serine proteases.[26−28] While FB can exhibit weak catalytic activity against small dipeptides and tripeptides in vitro,[29] in an endogenous setting, full catalytic competency requires an activation event, as is typically the case with chymotrypsin-like serine proteases. In the case of FB this involves binding to C3b, followed by FD mediated release of Ba as described earlier. These rearrangements, and the dissociation of Ba, cause significant conformational movement in the SPD, enabling access to substrate (i.e., C3), even though the orientation of the catalytic triad does not appear to be significantly altered during these events.[30−32] This mechanism is distinct from activation of more typical chymotrypsin-like serine protease zymogens. Generally, when the zymogen undergoes proteolytic activation, a free NH_3^+ terminus is formed to enable a salt bridge with a conserved Asp in the S1 substrate binding pocket. This event enables the catalytic site

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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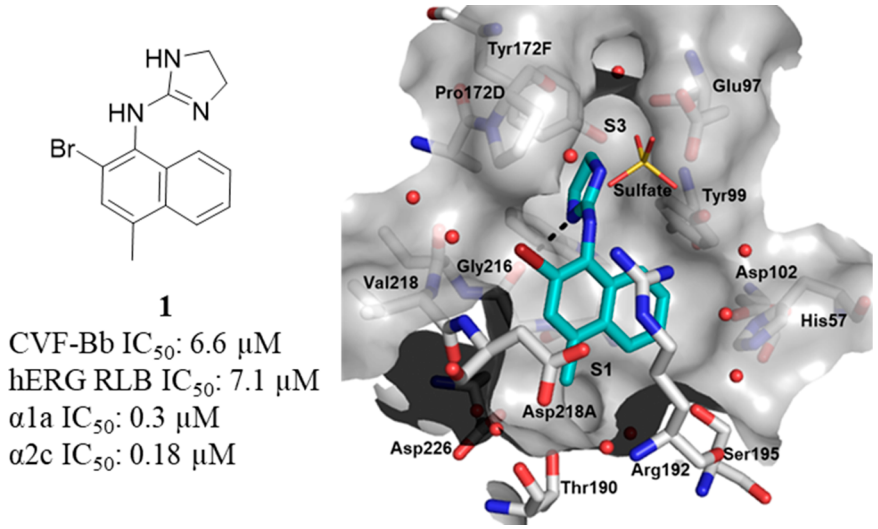

Figure 2. Cocrystal structure of compound **1** bound to the catalytic domain of human FB at 1.64 Å resolution (6QSW.pdb)[13] and in vitro potency against CVF-Bb, hERG, and adrenergic receptors α1a and α2c. The ligand binding pocket is shown in gray, and H-bonding interaction is shown as black dotted lines. Crystallographic water molecules are shown as red spheres.

to create an "oxyanion hole" and adopt an active confirmation.[33] FD mediated cleavage of C3bB does not liberate a free NH_3^+ terminus within the SP domain; thus the active site exhibits a different charge distribution than in other serine proteases.[34] Furthermore, FB differs from other trypsin-like serine proteases by having a neutral Asn at the bottom of the S1 pocket instead of the typically conserved negatively charged Asp (position 189; chymotrypsinogen numbering has been used throughout this paper) and a relatively larger Asp226 instead of a neutral glycine residue.[35] From a drug discovery perspective, we hoped to capitalize on the unique features of FB and its activation. We rationalized that the distinct SPD of FB might make protease selectivity less of a concern but would also suggest that using other serine protease inhibitors as chemical starting points may not be viable. Furthermore, the dramatic conformational changes that accompany FB binding to C3b and the loss of Ba via FD might provide an opportunity for molecules that have a preferential affinity for the C3 convertase SPD over the latent FB SPD, which could be advantageous considering the high abundance of latent FB in serum.

With these concepts in mind we undertook an aggressive integrated hit finding approach that included (1) a high throughput screen to evaluate activity of the full Novartis compound collection (~1.1 million compounds at that time) in a MAC deposition assay in an ELISA format, (2) a more FB targeted high throughput assay of ~250K compounds measuring inhibition of C3 cleavage by a form of FB activated by cobra venom factor (CVF-Bb),[36] and (3) a fragment based screen (FBS) guided by NMR and X-ray. While all three approaches afforded interesting chemical starting points, ultimately the CVF-Bb assay proved most fruitful affording a hit rate of ~0.6% and identifying the aminoimidazoline **1** (Figure 2). Compound **1** afforded a mean IC_{50} of 6.6 ± 3.5 μM (n = 22) in this assay format and was further validated biophysically by SPR (K_d = 10 μM) and via cocrystallization with the catalytic domain of human FB. Moreover, **1** afforded IC_{50} values of >30 μM when assessed against an internal panel of 17 proteases. The crystal structure shows the imidazoline ring positioned in the S3 pocket formed by residues Glu97, Tyr99, Tyr172F, Pro172D and making a hydrogen bond with the backbone carbonyl of Gly216 (Figure 2).[37] The naphthyl ring appears to establish a cation−π interaction with Arg192, perhaps stabilized in the crystal structure by the presence of a sulfate ion from crystallization buffer solution. The naphthyl resides at the opening of the S1 pocket. The bromine substituent at C-2 of the naphthyl occupied a groove formed by residues Pro172D and Val218.

While a cursory inspection of the cocrystal structure of **1** suggested many opportunities to enhance potency by expanding and/or filling regions of the protein (e.g., oxyanion hole and S1′ pocket), we initially chose to explore modifications of the imidazoline with an additional aim of improving the off-target selectivity profile of **1**. Profiling of **1** in an internal panel of over 25 enzymes, receptors, and ion channels highlighted undesirable activity against hERG (radioligand binding (RLB) assay IC_{50} = 7.1 μM) and binding to the adrenergic α1a and α2c receptors, with IC_{50} values of 0.3 and 0.18 μM, respectively (Figure 2). We rationalized that basicity of the imidazoline was the source of the observed off-target activity.[38,39] Before we began our efforts to replace the imidazoline we first replaced the bromine at the 2-position of the naphthyl ring of **1** with the more chemically stable chloride to afford **2** with similar potency against FB (CVF-Bb IC_{50} = 8.8 μM; Table 1). Turning to the imidazoline, replacement with less basic aminoimidazole derivative **3** led to a complete loss in activity. Reviewing the X-ray structure of **1** bound with FB, it appeared that the S3 pocket formed by residues Glu97, Tyr99, Tyr172F, and Pro172D would open up and accommodate larger ring systems, leading to the design of benzimidazole derivative **4**. However, trying to enhance potency by filling this pocket while reducing basicity also led to a complete loss in activity. More subtle modifications such as replacement of the imidazoline with the oxazoline isostere (**5**) or the slightly larger tetrahydropyrimidine (**6**) maintained some affinity for FB but were clearly inferior to the imidazoline.

With the initial relatively conservative efforts to replace the imidazoline not having proven productive, we hypothesized that alterations to the naphthyl core may provide the necessary enhancements in potency to warrant a re-evaluation of

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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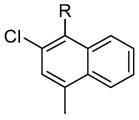
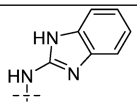
Table 1. SAR Exploration Related Replacement of the Imidazoline of Compound 2

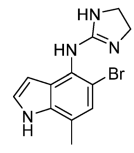
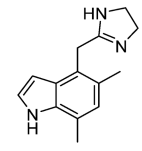
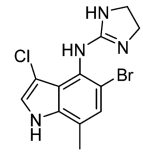
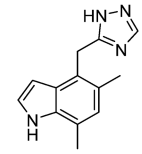
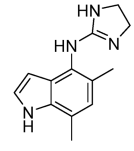
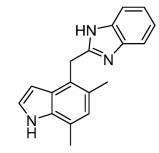
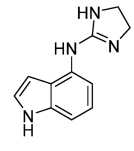
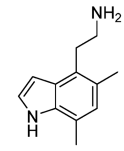
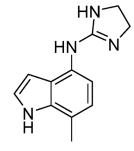
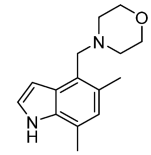
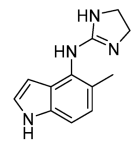
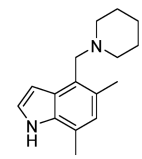
Compound	CVF-Bb IC_{50}[a] (μM)
	
 **2**	8.8
 **3**	> 100
 **4**	> 100
 **5**	33
 **6**	65

[a]Half-maximal inhibition of cobra venom factor Bb complex (CVF-Bb) in an ELISA format; data represent mean values of at least duplicate measurements.

imidazoline replacements. To that end we returned to the X-ray of **1** bound to FB to guide these efforts. Direct elaboration of the naphthyl ring did not appear promising when considering what appeared to be a relatively tight fit of the 2-bromine and 4-methyl substituents. The naphthyl core also did not offer tractable trajectories to grow the molecule into the oxyanion hole, a common means of enhancing potency.[40,41] In addition, the apolar nature of the naphthyl would potentially make it more challenging to create positive interactions with the relatively polar S1 pocket. However, the X-ray structure suggested that replacing the naphthyl core with an indole would be advantageous. We rationalized that the 2-position of the indole would offer the appropriate trajectory to vector toward the oxyanion hole, while the 3-position would offer an opportunity to fill the space near Tyr99. To explore these possibilities, we synthesized indole **7** (Table 2), which encouragingly maintained the potency observed for the direct naphthyl comparator **1**. To explore whether the C-3 position of the indole offered an opportunity to enhance potency, the 3-chloro indole **8** was prepared. While **8** afforded a modest drop in potency, it more importantly furnished a cocrystal structure with the catalytic domain of FB (Figure 3).

Surprisingly, the crystal structure of **8** revealed that the indole core adopts a flipped binding orientation relative to

Table 2. Initial SAR Exploration Related to Indole Derivative 7

Compound	CVF-Bb IC_{50} (μM)[a]	Compound	CVF-Bb IC_{50} (μM)[a]
 **7**	8.0	 **13**	6.3
 **8**	11	 **14**	>100
 **9**	6.6	 **15**	6.7
 **10**	>100	 **16**	>100
 **11**	>100	 **17**	>100
 **12**	>100	 **18**	50

[a]Half-maximal inhibition of cobra venom factor Bb complex (CVF-Bb) in an ELISA format; data represent mean values of at least duplicate measurements.

what was hypothesized. Instead of pointing toward Tyr99, the 3-chloro group followed the trajectory of the bromine atom of **1**, while the indole nitrogen established a hydrogen bond with the backbone carbonyl of Thr190, as opposed to being oriented closer to Ser195. In addition, the indole was positioned deeper in the S1 pocket than the naphthyl, even though the imidazoline maintained the H-bonding with Gly216. This unexpected binding mode led us to reassess the importance of the C-5 bromo and C-7 methyl on the indole core (Table 2). Replacement of the C-5 bromide with a methyl (**9**) afforded similar activity against FB. However,

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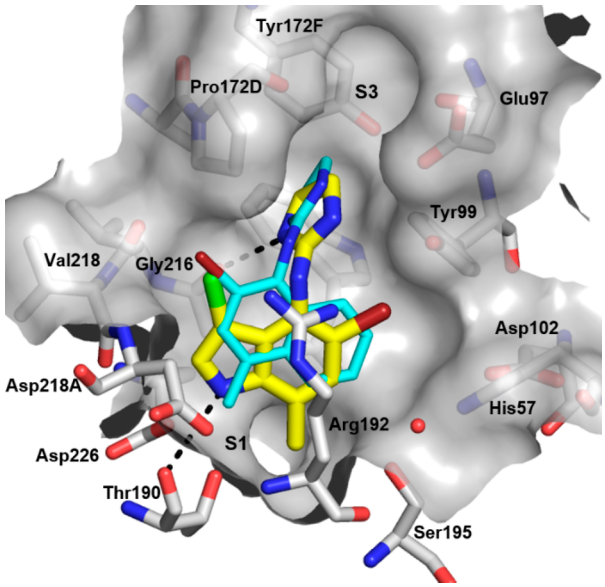


Figure 3. Cocrystal structure of indole **8** (yellow) with the catalytic domain of FB at 2.84 Å resolution (6T8U.pdb) overlaid with naphthyl **1** (cyan). The ligand binding pocket is shown in gray, and H-bonding interactions are shown as black dotted lines. Crystallographic water molecules are shown as red spheres.

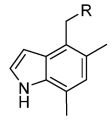
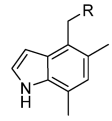
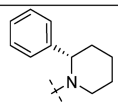
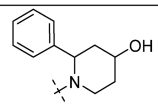
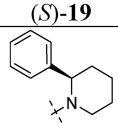
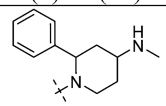
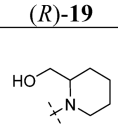
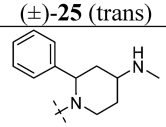
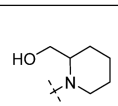
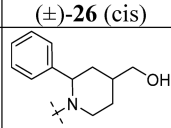
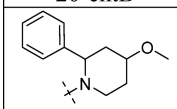
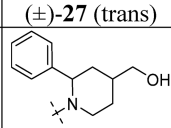
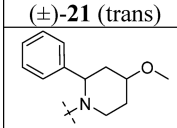
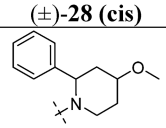
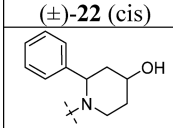
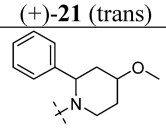
substitution at C-5 was found to be critical as compounds **10** and **11** did not exhibit any inhibitory activity up to 100 μM. Substitution at C-7 also proved to be critical with compound **12** also being devoid of appreciable activity against FB.

With the new indole core in hand, and a better understanding of the SAR at C-5 and C-7, we returned to our initial goal of replacing the aminoimidazoline, as indole **7** still exhibited binding to the α2c adrenergic receptor with an IC_{50} of 0.15 μM. However, the indole core did improve selectivity over hERG and the α1a adrenergic receptor with **7** possessing a hERG RLB IC_{50} of 24 μM, and an IC_{50} of >30 μM for α1a. A conservative replacement with a carbon linked imidazoline afforded **13** (Table 2) that maintained FB activity but did not attenuate α2c activity (0.38 μM) and brought back hERG and α1a activity with IC_{50} values of 7.2 μM and 0.49 μM, respectively. Nonetheless, we were encouraged by the fact that the 2-amino linker was not required for on-target affinity. However, replacing the imidazoline with the substantially less basic 1,2,4-triazole as in **14** led to a complete loss of FB activity. Recognizing that positioning of the indole core deeper in the S1 pocket might afford additional space in the S3 pocket, we reinvestigated the benzimidazole motif which was not suitable when combined with the naphthyl core. In the case of the indole core, the C-linked benzimidazole (**15**) proved much more fruitful. Compound **15** maintained FB activity relative to **13** but abolished α1a, α2c, and hERG activity (all >30 μM). This encouraging result led to a substantial SAR campaign aimed at optimizing the potency and ADME properties of this benzimidazole subseries, which is out of the scope of this manuscript and will be reported separately in due course. In addition to the benzimidazole we also explored simpler amines of varying basicity. Introduction of an ethylamine (**16**), aimed at mimicking one of the basic amines of the imidazoline, led to nearly complete loss in FB activity (~15% inhibition at 100 μM), as did the morpholine **17** (~21% inhibition at 100 μM). However, despite these results, the piperidine derivative **18** afforded a measurable IC_{50} of 50 μM. The simplicity and small size (MW = 242.4 g/mol) of **18** combined with the ability to replace the concerning aminoimidazoline moiety with a piperidine that offered new vectors to grow from led us to further explore the SAR around this compound.

Referring to the X-ray cocrystal structures of **1** and **8**, it appeared that the piperidine offered suitable vectors both to the S3 pocket and to a groove in FB between Pro172D and Val218 that is occupied by the Br of **1** and the Cl of **8**. In the probing of this groove, the 2-position of the piperidine was functionalized by phenyl (**19**) and $-CH_2OH$ (**20**) groups to explore the impact of lipophilic and polar functionalities (Table 3). Encouragingly, both modifications to the piperidine led to an improvement in FB potency with the stereochemistry playing a significant factor.

The more potent phenyl enantiomer yielded a cocrystal structure with FB (Figure 4), which enabled the determination

Table 3. SAR Related to the 2- and 4- Positions of the Piperidine Moiety

Compound 	CVF-Bb[a] or {FB-comp[b]} IC_{50} (μM)	Compound 	FB-comp IC_{50} (μM)[b]
 (*S*)-**19**	5.9	 (±)-**24** (cis)	>100
 (*R*)-**19**	>100	 (±)-**25** (trans)	36
 **20**-entA	6.0	 (±)-**26** (cis)	6.8
 **20**-entB	>100	 (±)-**27** (trans)	2.5
 (±)-**21** (trans)	{1.4}	 (±)-**28 (cis)**	7.3
 (±)-**22** (cis)	{11}	 (+)-**21** (trans)	68
 (±)-**23** (trans)	{14}	 (−)-**21** (trans)	0.72

[a]Half-maximal inhibition of cobra venom factor Bb complex (CVF-Bb) in an ELISA format. [b]Half-maximal inhibition of human FB in TR-FRET based competition binding assay (FB-comp); data represent mean values of at least duplicate measurements.

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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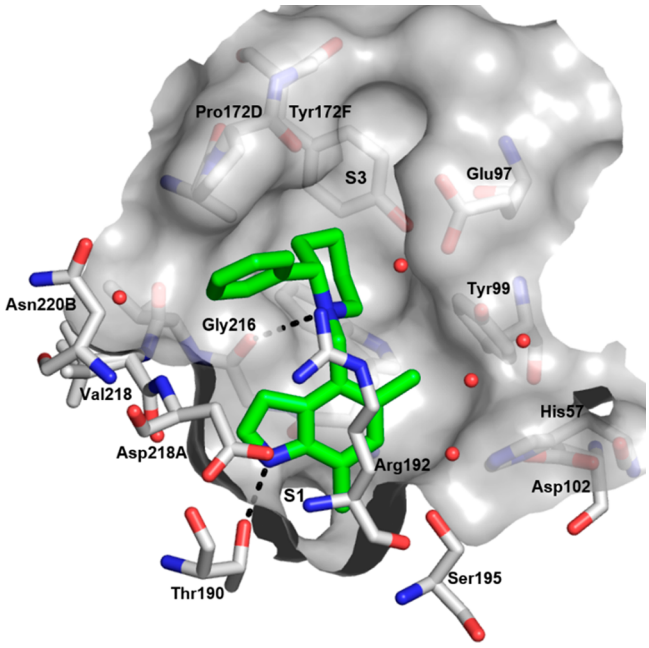
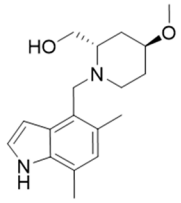


Figure 4. Cocrystal structure of indole (*S*)-**19** (green) with the catalytic domain of FB at 1.70 Å resolution (6T8W.pdb). Of note the phenyl ring sits in the Pro172D and Val218 groove. The ligand binding pocket is shown in gray, and H-bonding interactions are shown as black dotted lines. Crystallographic waters are shown as red spheres.

that the *S* absolute stereochemistry was preferred. The X-ray structure also revealed that indeed the phenyl ring situated nicely in the groove between Pro172D and Val218. The compound went deeper into the S1 pocket relative to **8** with the indole nitrogen now making a H-bond with the side chain of Thr190 instead of the backbone carbonyl in **8**. The protonated nitrogen of the piperidine appears to be making a H-bonding interaction with Gly216 similar to the imidazoline.

The X-ray also revealed that the 4-positon of the piperidine was well positioned to enable growing into the S3 pocket. Guided by the X-ray of (*S*)-**19**, we first decided to explore the S3 pocket with a small set of phenylpiperidines, before following up on the hydroxyl analog **20**. The selected disubstituted piperidines were prepared as racemic sets of diastereomers (**21**−**28**, Table 3), and the ascribed relative stereochemistry was elucidated by NOE interactions between C-2 and C-4 protons of the piperidines. Of note, at this stage in the program, we began to employ an alternative assay format for our primary FB biochemical screening assay, a more operationally efficient TR-FRET based competition binding assay (FB-comp). In this assay, compounds are evaluated for their ability to displace a Cy5-labeled small-molecule inhibitor probe from native FB.[42] Installation of an −OMe at the 4 position of the piperidine afforded a substantial improvement in potency when oriented in a trans configuration, and importantly (±)-**21** also exhibited a substantial reduction in α2c receptor binding affinity (IC_{50} = 11 μM), although hERG activity remained (RLB IC_{50} = 2.3 μM). Replacement of the ether with a hydroxyl ((±)-**23**) led to a ∼10-fold drop in potency. Interestingly, when the C-4 position was substituted with a methylamine, the stereochemical preference was reversed with the cis conformation proving more potent ((±)-**26**). Lastly, a $-CH_2OH$ moiety at C-4 reverted the preference to the trans configuration ((±)-**27**) and afforded only a slight reduction in potency relative to (±)-**21**. Considering the potency of (±)-**21** and the desire to avoid any potential ADME liabilities associated with the primary alcohol of (±)-**27**, we chose to assess the individual enantiomers of (±)-**21**. Compound (−)-**21** was determined to be the more active of the two enantiomers and for the first time in this subseries afforded a submicromolar IC_{50} value of 0.72 μM. The SAR learnings from the C-2 phenyl substituted piperidines also applied to the C-2 alcohol **20**, as the C-4 methoxy substituted piperidine (2*S*,4*S*)-**29** (Figure 5), which also afforded a ∼2× improvement in potency over the most active enantiomer of **20**.[43]

In addition to the enhancement in FB potency, the binding affinity of **29** against the α1a and α2c receptors was reduced with IC_{50} values of >30 μM and 16 μM, respectively. The hERG inhibitory activity (RLB IC_{50} = 14 μM) was also attenuated relative to (±)-**21**. More intriguingly, a cocrystal of (2*S*,4*S*)-**29** with FB revealed an interaction between the hydroxyl group and a sulfate ion from the crystallization buffer,



29
CVF-Bb IC_{50}: 3.35 μM
hERG RLB IC_{50}: 14 μM
α1a IC_{50}: >30 μM
α2c IC_{50}: 16 μM

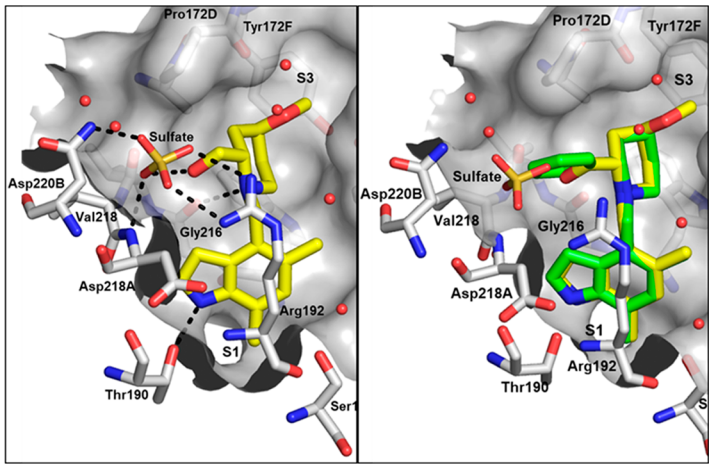


Figure 5. In vitro potency of (2*S*,4*S*)-**29** as assessed in the CVF-Bb assay, a cocrystal structure of (2*S*,4*S*)-**29** (yellow) bound to the catalytic domain of human FB at 1.77 Å resolution (6QSX.pdb)[13] highlighting an interaction with a sulfate ion from the crystallization buffer, and an overlay with (*S*)-**19** (green) from its cocrystal structure. The ligand binding pocket is shown in gray, and H-bonding interactions are shown as black dotted lines. Crystallographic water molecules are shown as red spheres.

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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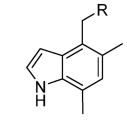
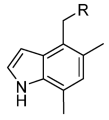
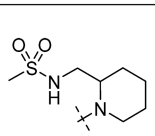
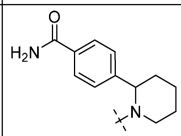
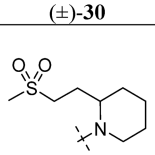
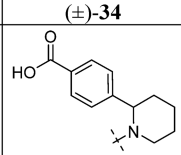
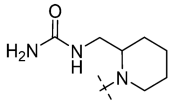
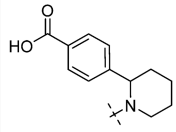
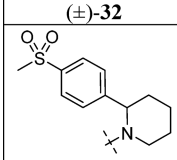
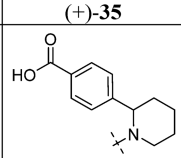
which in turn made a complex hydrogen bonding network with Arg192, Val218, and Asn220B (Figure 5). In an effort to capitalize on this observation, we tried to mimic this interaction by incorporating moieties at the C-2 position of the piperidine that had the potential to engage in a similar hydrogen bonding network as the sulfate ion (Table 4).

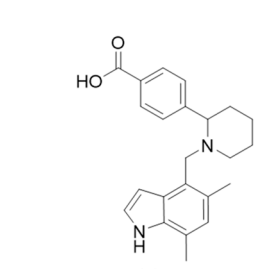
Table 4. SAR Related to Efforts To Mimic the Interactions of the Sulfate Ion Observed in the Cocrystal Structure of 29 with FB

Compound	FB-comp IC_{50} (μM)[a]	Compound	FB-comp IC_{50} (μM)[a]
			
 (±)-**30**	> 100	 (±)-**34**	0.71
 (±)-**31**	12	 (±)-**35**	0.062
 (±)-**32**	27	 (+)-**35**	17
 (±)-**33**	1.3	 (−)-**35**	0.033

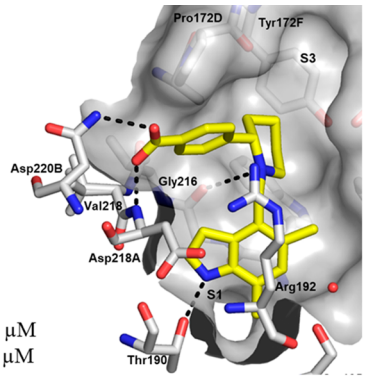
[a]Half-maximal inhibition of human FB in TR-FRET based competition binding assay (FB-comp); data represent mean values of at least duplicate measurements.

Unfortunately, attempts to replicate these interactions with a sulfonamide (**30**), sulfone (**31**), or urea (**32**) were largely unproductive, with only the sulfone affording potency in the range of **29**. These results led us to reassess the X-ray crystal structures of **29** and (*S*)-**19** bound to FB. When overlaying these two molecules (Figure 5), it became evident that the *para* position of the phenyl ring in (*S*)-**19** was perfectly situated to place a substituent into the space occupied by the sulfate. Thus, a series of *para* substituted phenyl piperidines were prepared with the aim of mimicking some of the protein interactions made by the sulfate ion (Table 4).

Sulfone ((±)-**33**) and amide ((±)-**34**) moieties both afforded a ∼4- to 8-fold improvement in FB potency as racemates relative to the unsubstituted enantiopure phenyl piperidine (*S*)-**19**. More strikingly, when a carboxylic acid was installed at the *para* position ((±)-**35**), a nearly 100-fold improvement in potency was observed, providing confidence that we were successfully mimicking at least to some extent the protein interactions seen with the sulfate ion. This was confirmed by X-ray crystallography as cocrystallization of (±)-**35** with FB yielded the structure shown in Figure 6. The



(−)-**35**
FB-Comp IC_{50}: 0.033 μM
50% hSerum MAC IC_{50}: 0.25 μM
50% mSerum C3b IC_{50}: 2.28 μM



In Vitro Properties		Pharmacokinetic Parameters 1 mg/kg i.v. and 10 mg/kg p.o.	
hERG RLB IC_{50} (μM)	>30	CL (mL/min/kg)	6.8
α1a IC_{50} (μM)	>30[a]	V_{SS} (L/kg)	3.1
α2c IC_{50} (μM)	>30	$T_{1/2}$ / MRT (h)	4.9 / 2.3
HT-$Sol_{6.8}$ (mM)[b]	0.98	$AUC_{0\text{-last}}$ p.o. (nM·h)[d]	17100
Ms / H LM[c] Cl_{int} (μL/min/mg)	<3.4 / 54.1	C_{max} (nM)	5410
Caco-2 Papp A-B x10^{-6} (cm/s) [B-A/A-B]	16.6 [0.6]	%F	47

Figure 6. Cocrystal of (−)-**35** (yellow) bound to the catalytic domain of human FB at 2.29 Å resolution (6T8V.pdb) in addition to in vitro potency, ADME, and mouse PK profile of (−)-**35**. [a]Determined for (±)-**35**. [b]Equilibrium high throughput solubility in aqueous buffer (pH 6.8). [c]Mouse (Ms) and human (H) liver microsomes (LM). [d]Plasma exposure was measured out to 7 h postdose.

structure revealed the acid to be engaged in several key hydrogen bonds to both Asn220B and the Asp218A-NH. Separation of the enantiomers of the carboxylic acid revealed (−)-**35** as the more potent enantiomer affording an IC_{50} of 0.033 μM.[44] Furthermore, the addition of the acid abolished hERG and adrenergic receptor activity. With this exciting improvement in potency and a clean off-target profile, more advanced profiling of (−)-**35** was warranted. In a functional in vitro assay assessing AP-mediated MAC deposition of 50% human serum induced by zymosan-A, (−)-**35** afforded an IC_{50} of 0.25 μM. The submicromolar IC_{50} value was of particular note as this concentration is significantly below the endogenous FB protein levels of ∼1 to 2 μM in these diluted human samples. This result is indicative of (−)-**35** perhaps having an affinity preference for the FB SPD when part of C3 and/or C5 convertase complex (vide supra). Acid (−)-**35** also possessed excellent physicochemical and ADME properties, which translated to an attractive mouse pharmacokinetic profile (Figure 6).

Unfortunately, (−)-**35** proved to be substantially less efficacious when assessed for functional activity using a 50% mouse serum assay measuring C3b deposition (IC_{50} = 2.28 μM), presumably due to species differences in the protein structure. From sequence analysis, we determined that the catalytic domain of mouse FB has a sequence identity of only 80% compared to human. In particular, three residues,

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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Pro172B (Gln in mouse FB), Arg192 (Lys in mouse FB), and Asn220B (Asp in mouse FB) that form the ligand binding pocket are different in mouse FB. While potency against human FB was obviously most critical, the fact that the in vivo pharmacology of a selective FB inhibitor had never been reported led to the desire to identify compounds that were potent against both the human and mouse protein to enable evaluation of PK/PD and efficacy in various disease models. We reasoned that filling the S3 pocket should provide an enhancement in potency against the human enzyme as was the case with (−)-**21** and **29** and that this may also lead to a concomitant increase in mouse potency. Installing the −OMe at C-4 of the piperidine yielded **36** possessing the 2*S*,4*S* stereochemistry as the most potent stereoisomer affording an IC_{50} of 0.024 μM in the human FB-comp assay (Table 5).[45]

Table 5. In Vitro and in Vivo Profiles of Advanced FB Inhibitors

Compound	FB-Comp IC_{50} (μM)[a]	50% human / mouse serum IC_{50} (μM)[b]	Mouse plasma PD[c] inh.@8h (30 mg/kg)	Mouse ocular PD[c] inh.@8h (30 mg/kg)	Mouse PK Parameters[d] CL/Vd_{ss} $T_{1/2}$ / MRT $AUC_{0\text{-last}}$/%F
R = Me Z= Me **36**	0.024	0.15 / 0.16	43%	35%	8 / 4.3 6.6 / 2.1 10.8 / 41
R = Me Z= cPr **37**	0.018	0.24 / 0.12	35%	43%	10 / 4.4 5.6 / 2.4 9.8 / 83
R = Me Z= OMe **38**	0.018	0.18 / 0.15	85%	60%	14.3 / 1.8 2.4 / 1.4 7.7 / 37
R = Et Z= Me **39**	0.018	0.11 / 0.12	47%	10%	N/A
R = Et Z= cPr **40**	0.006	0.14 / 0.08	71%	23%	3.2 / 3 9.6/3.3 5.4 / 15
R = Et Z= OMe **41**	0.012	0.13 / 0.11	83%	83%	16 / 6.6 7.2 / 1.6 7.6 / 46

[a]IC_{50} values are mean values for at least $n = 2$. [b]For human, serum IC_{50} values reflect inhibition of MAC deposition, and for mouse C3b deposition, all values are mean values of at least $n = 2$. [c]Assessments of the inhibition of C3d+iC3b formation in the mouse LPS-challenge model. [d]Mice were dosed at 1 mg/kg iv and 10 mg/kg po, and compound exposure was measured in plasma. CL is represented in mL min^{-1} kg^{-1}. Vd_{ss} is expressed as L/kg. $T_{1/2}$ and MRT are expressed in hours, and $AUC_{0\text{-last}}$ is derived from po dosing and represented in μM·h, with the last plasma measurement taken at 7 h postdose.

More importantly **36** afforded similar potency in both the 50% human serum MAC deposition assay and the 50% mouse serum C3b deposition assay with IC_{50} values of 0.15 μM and 0.16 μM, respectively. In addition, **36** afforded a promising mouse PK profile demonstrating low clearance (8 mL min^{-1} kg^{-1}) and good oral bioavailability (41%).

The improvement in potency in mouse serum along with an acceptable PK offered the opportunity to evaluate **36** in a PD model of AP complement activation.[9] The model involved an intraperitoneal administration of lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, which induces activation of the AP. Activation can be assessed by the measurement of an increase in AP breakdown products, in particular C3d and iC3b. Proteolysis of C3b, the product of C3 convertase activity, by factor I (FI) with factor H (FH) serving as a cofactor generates iC3b. iC3b can be further broken down by FI into C3c and C3d (Figure 1). Thus, a reduction in C3d+iC3b levels of LPS treated mice upon FB inhibitor administration would afford an assessment of AP inhibition. Importantly, in mice treated with intraperitoneal LPS, AP activation is observed both in plasma and in ocular tissues, thus providing an opportunity to assess PD in both compartments. Encouragingly, compound **36** administered orally at a dose of 30 mg/kg provided an inhibition of C3d +iC3b levels at 8 h postdose of 43% and 35% in plasma and in ocular tissues (whole eye homogenate), respectively. The promising in vivo profile for **36** led us to evaluate subtle changes to the scaffold, in particular by expanding deeper into the S3 pocket via replacement of the C-4 piperidine methoxy with an ethoxy residue, as well as probing the small space occupied by the indole C-5 methyl in an effort to maximize van der Waals interactions with the protein (Table 5). These subtle changes had very little impact on the in vitro FB potency of the compounds as assessed in either the FB-comp biochemical assay or the functional serum assays. Furthermore, these subtle changes only led to modest changes in relevant PK parameters with no apparent SAR trends observed. However, despite the similar in vitro potency and PK profiles, the compounds did differentiate themselves in terms of in vivo PD, in particular in ocular tissues. Empirically it was observed that indoles substituted with a C-5 methoxy (e.g., **38** and **41**) afforded a greater degree of ocular AP suppression than methyl and cPr comparators. The methoxy substituted indoles also provided better suppression of in vivo plasma AP activation as well, although the differences compared to the corresponding cPr and methyl analogs were not as dramatic. Compound **41** demonstrated the best balance of plasma and ocular inhibition and was therefore selected for further profiling.

From an in vitro perspective, **41** exhibited a K_d value of 7.9 nM with human FB as determined by SPR. The X-ray cocrystal of **41** with FB protein (Figure 7)[46] revealed the acid hydrogen bonding with Asn220B side chain and the Asp218A-NH and the ethoxy group positioned in the S3 pocket, while the indole nitrogen maintained an interaction with Thr190 and the indole methoxy occupying a small pocket formed by His57 and Tyr99.

Moreover, **41** exhibited excellent selectivity over other proteases affording IC_{50} values of >30 μM across a panel of 41 human proteases, including the AP protein factor D (>100 μM). The selectivity was consistent with the unique nature of the FB SPD. Additionally, **41** furnished IC_{50} values of >30 μM across an internal panel of ∼110 enzymes, receptors, and ion channels, including hERG and adrenergic receptors α1a and α2c.[47]

In vivo, **41** exhibited a dose responsive relationship for plasma and ocular inhibition of iC3b+C3d when employed in the C57BL/6 mouse LPS challenge mouse model at doses ranging from 3 to 30 mg/kg when assessed at 4 h (Figure 8).[48] A full time course of a single 30 mg/kg oral dose indicated near complete AP inhibition out to 8 h in both plasma and ocular tissues with partial suppression of iC3b+C3d in both tissues out to 12 h.

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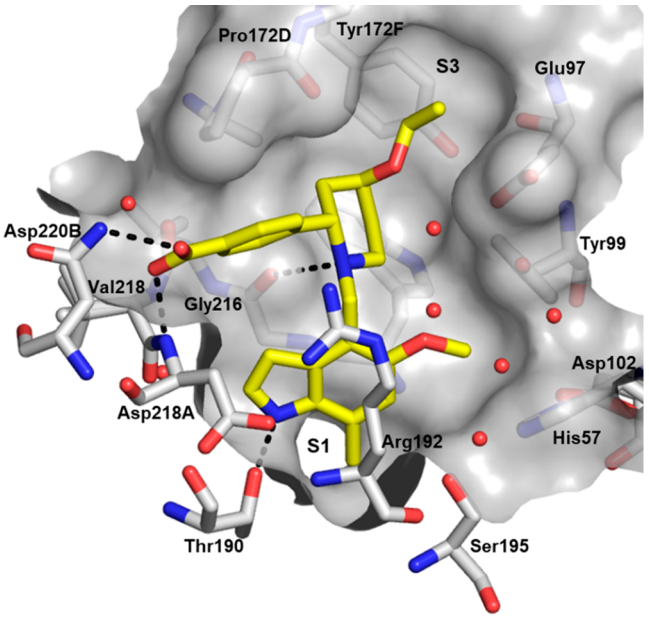

Figure 7. Cocrystal of **41** within the catalytic domain of FB at 1.7 Å resolution (6RAV.pdb).[13] The ligand binding pocket is shown in gray, and H-bonding interactions are shown as black dotted lines. Crystallographic waters are shown as red spheres.

The observed pharmacology in whole mouse eye homogenates was promising as it relates to the potential of developing a FB inhibitor as a therapy for AMD. In AMD it is thought that the disease may initiate at tissues of the posterior segment, in particular in the RPE, Bruch's membrane (BM), and choroid region.[49−51] Unfortunately, the small size of the mouse eye hampers precise tissue dissection, making a direct assessment of PK/PD in RPE/BM/choroid tissues particularly challenging. Thus, to better understand the exposure of **41** in these tissues, we conducted ocular PK studies in pigmented brown Norway rats (Figure 9). Upon an oral dose of 10 mg/kg, exposure was evaluated in the posterior eye cup (PEC), which consists of the target tissue, the RPE/BM/choroid, and the posterior sclera. Exposure in the retina and plasma was also evaluated. In brown Norway rats, **41** achieved exposure in the PEC well above the IC_{50} value recorded in the 50% human serum MAC deposition assay, and while the C_{max} in this compartment was blunted relative to what was observed in plasma, there was an enhancement in AUC, potentially due to **41** exhibiting a moderate affinity for melanin.[52,53] Exposure in the retina was substantially lower presumably due to limited ability to cross the BRB.[22]

With a better understanding of the ocular PK profile of **41**, we then looked to further characterize the systemic PK profile in species beyond the mouse (Figure 10). The iv/po dosing experiments with **41** in Wistar Han rats and beagle dogs demonstrated that low clearance and good oral bioavailability were not limited to mice. Plasma protein binding was also comparable across species with values ranging from 57% to 74% in mouse, rat, dog, and human plasma at a concentration of 5 μM of **41**. The robust PK profile in the dog also enabled evaluation of the pharmacodynamic effects of **41** in an ex vivo

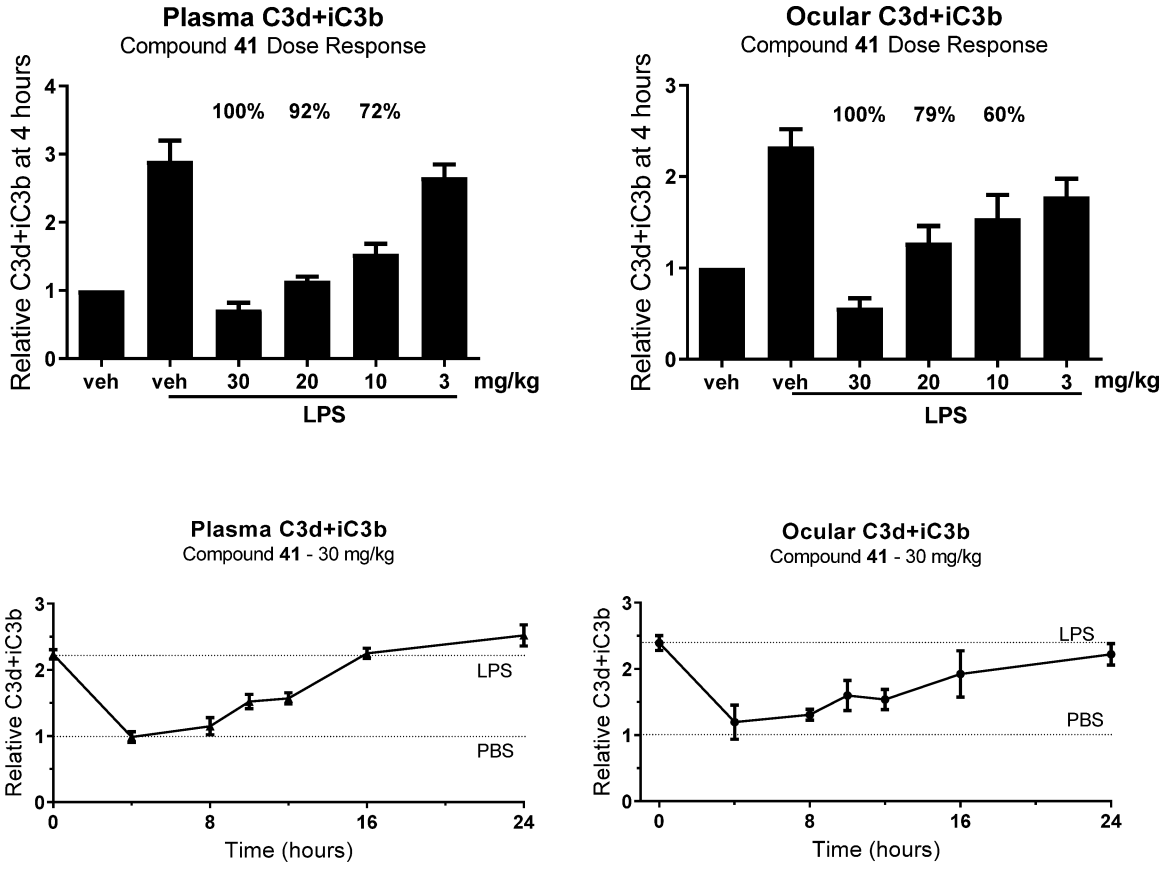

Figure 8. Dose−response relationship of **41** in the mouse LPS challenge model (% inhibition at 4 h ± SEM; all values have p values of <0.05) and time course of plasma[13] and ocular AP inhibition ± SEM with a 30 mg/kg oral dose of **41**.

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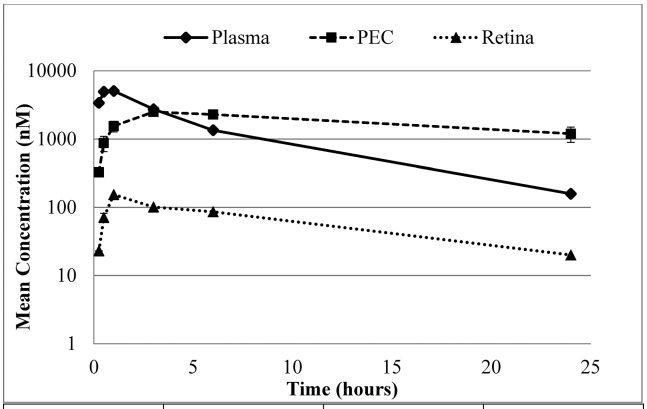

Parameters	PEC Mean	Retina Mean	Plasma Mean[a]
$AUC_{0\text{-last}}$ (nM*h)[b]	76100	1670	28600
C_{max}(nM)	2500 ± 170	150 ± 15	5100

Figure 9. Concentration versus time profile for **41** in PEC, retina, and plasma and PK parameters after a single dose (po 10 mg/kg) in brown Norway rats. [a]Plasma samples were taken only from two animals, and thus a standard deviation was not calculated. [b]AUC values were calculated as the mean of all the available concentration vs time data points for each ocular tissue. Standard deviations cannot be calculated because each time point is terminal.

dog experiment. In this study, **41** was dosed orally at a relatively low dose of 3 mg/kg and blood was drawn at various time points over the course of 36 h. The collected blood samples were processed to provide serum which was treated with zymosan-A to induce AP activation. Under these conditions **41** afforded complete suppression of AP activation, as measured by MAC deposition, out to 6 h and afforded 55% inhibition out to 12 h.

Compound **41** was also profiled in a series of experiments to assess efficacy of FB inhibition in various disease states including a mouse model of antibody-induced arthritis and a rat model of membranous nephropathy. In both cases **41** demonstrated both robust PD and efficacy as has been previously reported.[13] Compound **41** also demonstrated the ability to block AP activation ex vivo using blood from patients with C3G and PNH as has been previously disclosed.[13] C3G, a chronic kidney disease, is caused by C3 convertase dysregulation due to the presence of C3 convertase stabilizing autoantibodies (C3 nephritic factors) and/or mutations in complement proteins. PNH is characterized by the aberrant complement-mediated lysis of erythrocytes due to a somatic mutation in the PIG-A gene in hematopoietic stem cells leading to a lack of complement regulators on the cell surface. As previously reported, with C3G patient samples **41** could achieve statistically significant inhibition at a concentration of 1.1 μM, but concentrations of >3 μM were required for complete inhibition likely due to the high level of AP activity in patient sera.[13] In the case of PNH patient derived samples, **41** was effective in preventing erythrocyte lysis with an IC_{50} value of 0.4 μM.

On the strength of these data, **41**, possessing the internal compound code LNP023, was selected for IND-enabling toxicology studies in rats and dogs, where **41** afforded a safety profile that supported continued clinical development. Completion of Ph1 studies in healthy volunteers has led to the initiation of a series of ongoing Ph2 studies in patients with C3G, PNH, and IgA nephropathy, the results of which will be reported in due course.[54]

CHEMISTRY

Initial efforts to develop SAR around the aminoimidazoline hit **1** relied heavily on the use of the reaction of chloro-1,3-dimethylimidazolinium chloride (DMC-Cl) with the appropriate thiourea intermediate as outlined in Scheme 1.[55,56]

For exploration around the imidazoline ring, substituted naphthylanilines of type **A** were converted to thiourea intermediates of type **B**, followed by DMC-Cl mediated condensation with the appropriate diamine or intramolecular cyclization with the pendant R_2 group to provide the final heterocyclic compounds (see Supporting Information and Scheme 1, for additional details). Indoleimidazolines **7**–**12** were generally prepared by similar sequences: tosyl-protected indoles **C** were converted to thioureas **D**, followed by condensation with ethylenediamine, deprotection, and optional C3-functionalization (see also Supporting Information and Scheme 1).

Compounds that contain carbon-linked heteroaryls or nitrogen-containing aliphatic heterocycles at the indole 4-position were generally prepared as outlined in Scheme 2, taking advantage of the common aldehyde intermediate (**43**).

The common aldehyde intermediate was derived by employing a Sandmeyer reaction to convert amine **42** to the corresponding aryl iodide, which was then treated with *n*-butyllithium and DMF to provide **43**. To access imidazoline **13** and benzimidazole **15**, the aldehyde was converted to dibromoolefin **44** followed by condensation with the appropriate diamine. Alternatively, nucleophilic attack of **43** with the lithiate of SEM-protected 1,2,4-triazole gave alcohol **45**, which was reduced and deprotected to provide **14**. The aldehyde could also be used directly to make final compounds by reductive amination followed by deprotection, as was done for piperidine **18**. However, it was found to be more efficient to convert the aldehyde to chloride **46** and displace with the appropriate amines. Morpholine **17** and substituted piperidines **19**–**36** were all made using this strategy, in some cases requiring additional functionalization steps after the coupling to obtain the desired piperidine substitution patterns. The amine coupling partners were either commercially available (**17**, **19**, **20**), previously reported (**29**),[13] or synthesized in a similar manner to the route shown in Scheme 3 (see Supporting Information for additional details). For 2,4-disubstituted piperidines, the diastereomers could be separated by flash chromatography at various stages of the syntheses and were then carried forward separately to the final compounds as racemates. For select compounds, chiral HPLC or SFC was used to resolve the single enantiomers, either on the final compounds or on a protected late-stage intermediate.

A representative synthesis of the 2,4-disubstituted piperidine building block and its elaboration into final analogs **39**–**41** is shown in Scheme 3.

Synthesis of the requisite piperidines began with turbo Grignard-mediated addition of 4-bromobenzonitrile to 4-methoxypyridine followed by trapping with Cbz-Cl to provide dihydropyridone **47**. Sequential reductions afforded the 4-hydroxypiperidine as a mixture of diastereomers, which proved difficult to separate. However, conversion to the TBPDS silyl ether allowed for easier separation of the diastereomers by flash chromatography, and the desired trans diastereomer **48**

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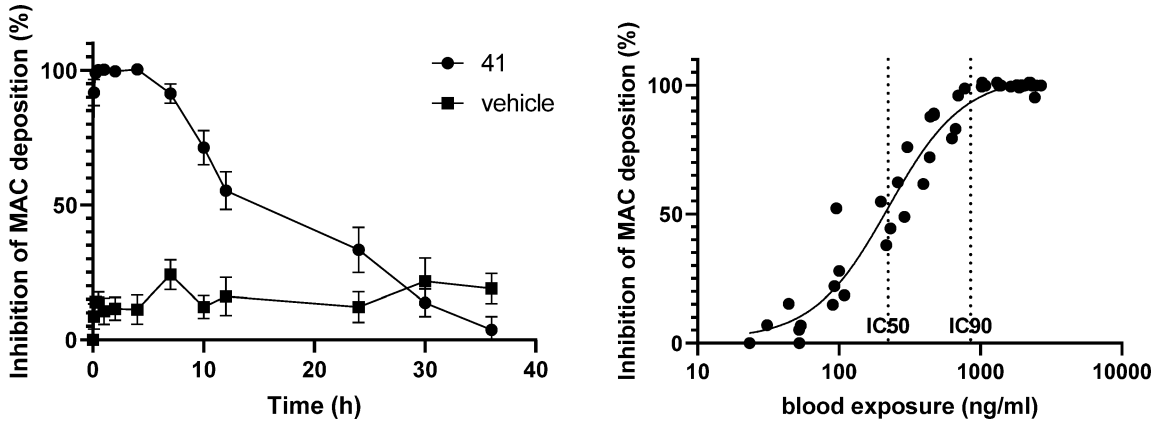
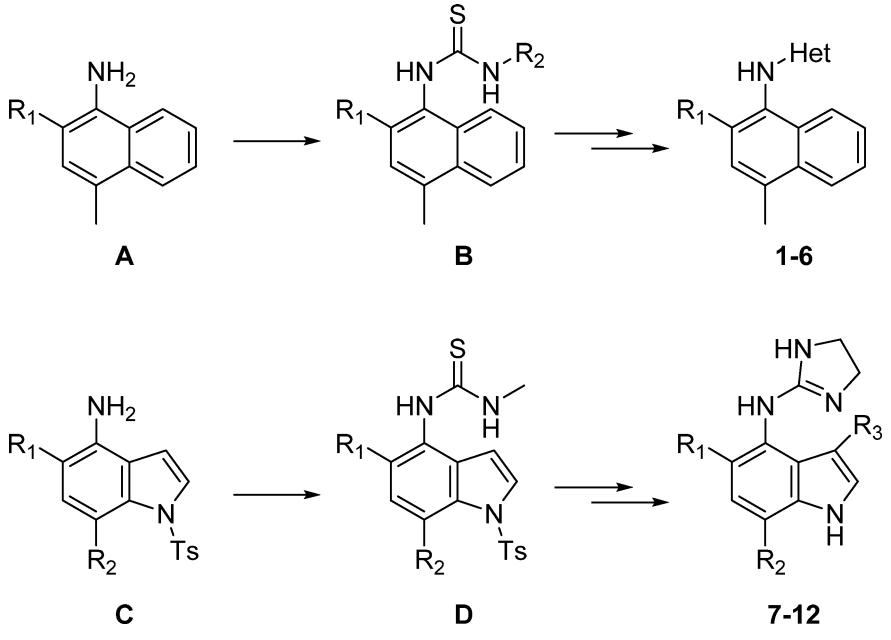


Parameters	Wistar-Han Rat	Beagle Dog
Dose i.v/p.o (mg/kg)	1 / 30	0.1 / 10
CL (mL/min/kg)	8	2 ± 0
Vd_{ss} (L/kg)	2.3	0.6 ± 0
T 1/2 / MRT (h)	7 / 3.4	5.6 ± 0.5 / 5.5± 0.3
AUC i.v. d.n. (nM*h)	5200	21000 ± 2200
AUC p.o. d.n. (nM*h)	2100	12700 ± 1100
F (%)	40	60 ± 5.5
C_{max} d.n. (nM)	410	2200 ± 300
T_{max} (h)	2	0.8± 0.3

Figure 10. Ex vivo pharmacodynamic effects of **41** in beagle dogs (n = 4, 3 mg/kg po), evaluating percent inhibition of MAC deposition in 50% serum ± SEM and PK/PD relationship, and pharmacokinetic parameters of **41** in Wistar Han rats (n = 2, at 1 mg/kg iv and 30 mg/kg po) and beagle dogs (n = 3, 0.1 mg/kg iv and 10 mg/kg po) and represented as mean values. d.n. denotes dose normalized. Standard deviations are reported wherever applicable.

Scheme 1. General Syntheses of Aniline-Linked Inhibitors 1−12



was then deprotected to give **49** as a racemic mixture. The required amine coupling partner **50** could then be accessed through standard functional group manipulations. **50** was reacted with indole chlorides **51** and **52** (prepared as described

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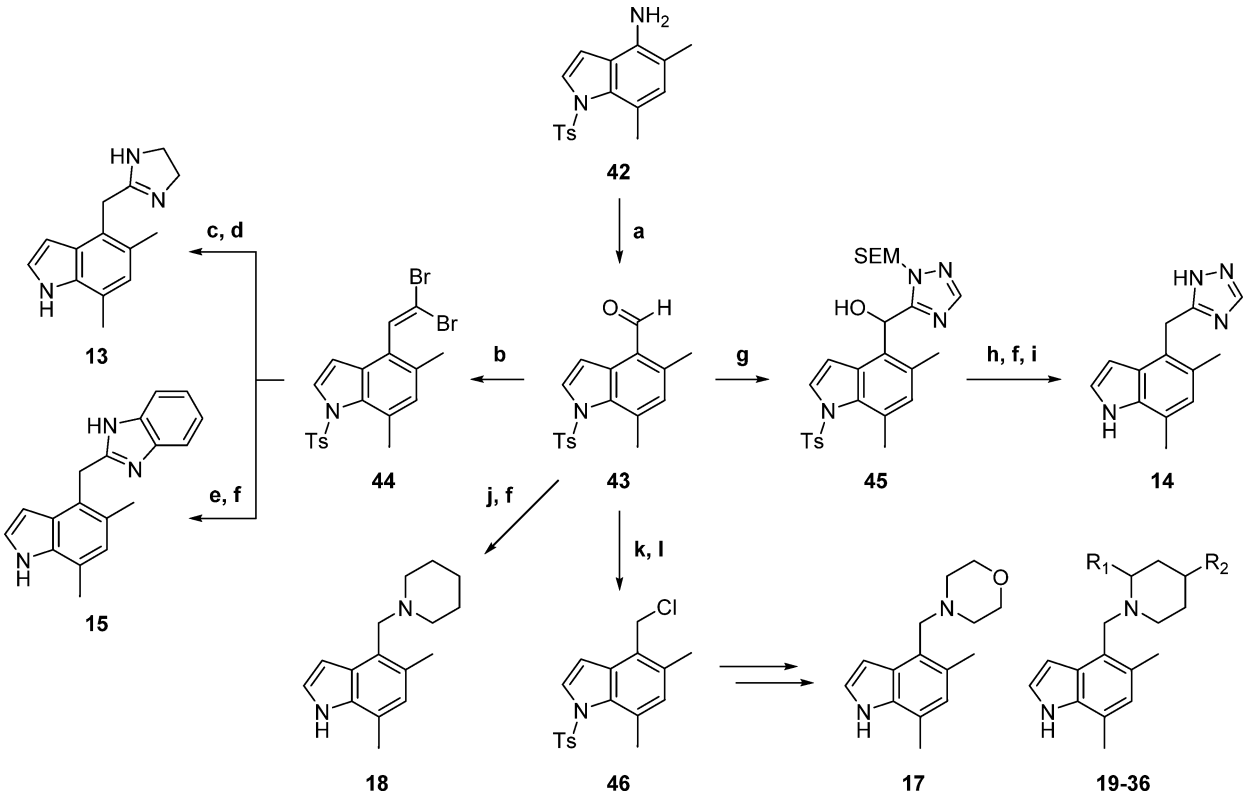
Scheme 2. General Synthetic Routes for C-Linked Indoles[a]



[a]Reagents and conditions: (a) $NaNO_2$, KI, HCl, H_2O, EtOAc; then *n*-BuLi, DMF, CPME; (b) CBr_4, PPh_3, DCM; (c) ethylenediamine; (d) isoamylamine, NaOEt, EtOH; (e) 1,2-phenylenediamine, DABCO, NMP; (f) LAH, THF; (g) 1-SEM-1,2,4-triazole, *n*-BuLi, THF; (h) MsCl, Et_3N, DCM; (i) TBAF, THF; (j) piperidine, $NaBH(OAc)_3$, AcOH, DCM, MeOH; (k) $NaBH_4$, MeOH, THF; (l) Vilsmeier reagent, DCM.

in Supporting Information) to generate protected intermediates **53** and **54**. Boc deprotection using K_2CO_3 in MeOH also caused partial hydrolysis of the methyl ester, which was fully re-esterified using TMS-diazomethane. We often found that the chiral separation of final compounds was quite challenging presumably due in part to their zwitterionic nature, so typically the separation was performed at the methyl ester stage followed by hydrolysis of each isomer, as was done for **39** and **40**. For **41**, we desired a route that would avoid such a late stage chiral separation, especially when working on larger scale. Therefore, intermediate **49** was resolved into its single enantiomers by chiral SFC, and the desired (2*S*,4*S*) enantiomer was converted to (2*S*,4*S*)-**50** in a similar manner as before. This was subjected to a reductive amination with aldehyde **57** (prepared as described in Supporting Information), followed by global deprotection with lithium hydroxide to complete an efficient synthesis of **41**.

CONCLUSIONS

An integrated hit finding approach identified **1**, containing a naphthyl core with an aminoimidazoline moiety, as a selective FB inhibitor. Knowledge gained from X-ray cocrystallography informed our efforts to enhance the potency and drug-like properties of **1**, resulting in replacement of the naphthyl core with an indole. X-ray cocrystallography further revealed that the indole scaffold bound to FB with a flipped binding orientation relative to **1** and offered new SAR opportunities in relation to exploring a groove in FB between Pro172D and Val218 and the S3 pocket leading to the piperidine **29**. Leveraging the serendipitous interaction of the hydroxyl of **29** with a fortuitous sulfate ion observed in a X-ray cocrystal with FB enabled a potency breakthrough by incorporating a phenylcarboxylic acid at the 2-position of the piperidine, which ultimately led to the discovery of **41** (LNP023), the first reported highly selective inhibitor of FB. Interestingly, **41** demonstrates an IC_{50} value in 50% human serum AP activation assay that is well below the concentration of FB, suggestive that **41** has preferential affinity for the SPD of FB when engaged as part of the C3 convertase complex. Compound **41** is orally bioavailable exhibiting low clearance across three preclinical species and achieves robust exposure in the posterior eyecup of rats. The potency and PK profile of **41** have led to the establishment of PD modulation and/or efficacy in several diverse animal models as well as in blood samples from patients with AP-mediated diseases. Compound **41** is currently undergoing Ph2 clinical evaluation in patients with C3G, PNH, and IgA nephropathy.

EXPERIMENTAL SECTION

1. Experimental Procedures and Compound Characterization for Novel Compounds. General Chemistry Information. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further drying or purification. NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. The following abbreviations are used to denote signal patterns: s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad. Unless otherwise noted, flash chromatography was conducted using grade 60 230−400 mesh silica gel from Fisher Chemical (S825-1) or by utilizing the CombiFlash Companion from Teledyne Isco, Inc. and RediSep Rf disposable

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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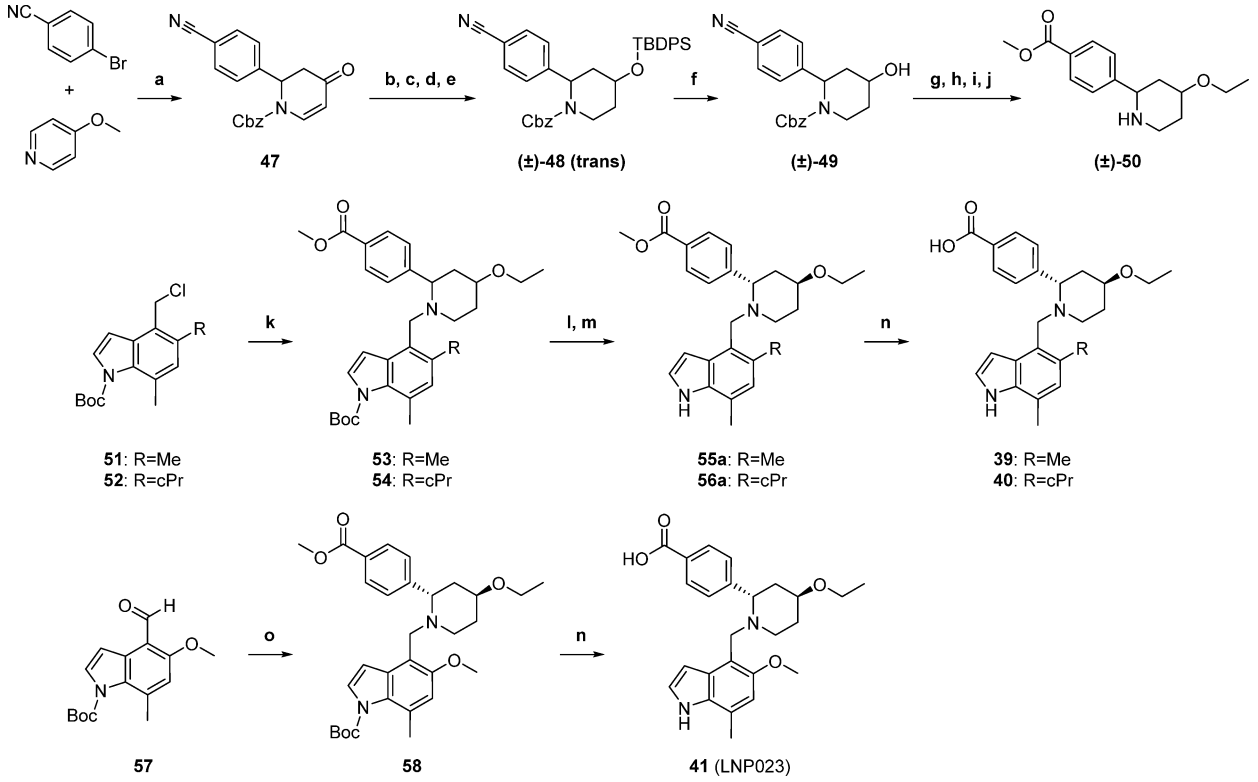
Scheme 3. Representative Synthesis of 2,4-Disubstituted Piperidine Intermediates and Inhibitors 39−41[a]



[a]Reagents and conditions: (a) ^{i}PrMgCl·LiCl, Cbz-Cl, THF; (b) Zn, AcOH; (c) $LiBH_4$, THF; (d) TBDPS-Cl, imidazole, DMF; (e) separation of diastereomers by flash chromatography; (f) TBAF, THF; (g) NaH, EtI, DMF; (h) $Ba(OH)_2$, ^{i}PrOH, H_2O; (i) K_2CO_3, MeI, DMF; (j) H_2, Pd/C, MeOH; (k) (±)-**50**, DIPEA, DMA; (l) K_2CO_3, MeOH; then TMS-diazomethane, toluene, MeOH; (m) chiral SFC; (n) LiOH, H_2O, MeOH, THF; (o) (2*S*,4*S*)-**50**, $NaBH(OAc)_3$, DCE.

normal phase silica gel columns (4−330 g). Thin layer chromatography was performed using 2.5 cm × 7.5 cm glass-backed TLC silica gel 60 F254 plates from EMD Chemicals, Inc. (15341-1) and visualized by UV light. HPLC purifications were performed on a Waters preparative HPLC system controlled by MassLynx. Systems were run with acetonitrile/water gradient with 0.1% TFA modifier on a Waters SunFire Prep C18 OBD 5 μm, 30 mm × 100 mm column (RP-HPLC-A) or 0.1% (28% aq NH_4OH) modifier on a Gemini NX 5 μm C18 110 Å 100 mm × 30 mm column (RP-HPLC-B). Low resolution mass spectra were recorded using an Agilent 1100 series LC−MS spectrometer. The purity of all exemplified compounds was ≥95%, as determined by both ^{1}H NMR and HPLC−UV at a wavelength of 214 nm. Optical rotations were obtained with a PerkinElmer model 341 polarimeter. Unless otherwise stated, chiral starting materials were commercially available with ee ≥ 98%. Compound **10** (CAS no. 67830-86-8) has been previously reported.[57] Synthetic procedures for indole and piperidine intermediates not described below can be found in the Supporting Information.

General Procedure A: Aromatic Halogenation. To a solution of the aromatic compound (1.0 equiv) in DMF (0.1 M) at −20 °C was added NCS or NBS (1.0 equiv), and this was warmed to 0 °C over 30 min. The reaction was diluted with saturated aqueous sodium bicarbonate and saturated aqueous sodium thiosulfate, extracted with EtOAc, dried with $MgSO_4$, filtered, and concentrated. The crude material was purified by FCC to provide the desired product.

General Procedure B: Thiourea Formation. To a solution of the aromatic amine (1.0 equiv) in DCM (0.1−0.25 M) at rt was added DIPEA or Et_3N (2.0−4.0 equiv) followed by thiophosgene (1.0−1.5 equiv). This was stirred for 15−60 min, then the aliphatic amine (2.0−8.0 equiv) was added and the mixture was stirred for 30 min. The reaction was diluted with DCM, washed with saturated aqueous sodium bicarbonate and saturated aqueous brine, dried with Na_2SO_4, filtered, and concentrated. The crude material was used without further purification or purified by FCC to provide the desired product.

General Procedure C: Guanidine Formation/Cyclization with DMC-Cl. To a solution of the thiourea (1.0 equiv) in ACN (0.05−0.2 M) at rt were added DIPEA or Et_3N (2.0−4.0 equiv) and chloro-1,3-dimethylimidazolinium chloride (DMC-Cl) (1.2−1.5 equiv). This was stirred at rt for 30−60 min, then the amine or diamine (2.0−5.0 equiv) was added, and this was stirred at reflux for 4−16 h. At this point the reaction mixture was diluted with DCM, washed with saturated aqueous sodium bicarbonate and saturated aqueous brine, dried with Na_2SO_4, filtered, and concentrated, or the reaction mixture was concentrated directly. The crude material was purified by FCC and/or RP-HPLC to provide the desired product.

General Procedure D: Nitro to Amine Reduction. A suspension of the nitro compound (1.0 equiv) and 10% Pd/C or PtO_2 (0.1 equiv) in MeOH or 1:1 MeOH/EtOAc (0.12 M) was stirred under hydrogen atmosphere at rt for 3−6 h. The reaction was filtered through Celite and concentrated to give the crude material, which was either used without further purification or purified by FCC to provide the desired product.

General Procedure E: Tosyl Deprotection with LAH. To a solution of the tosyl-protected indole (1.0 equiv) in THF (0.03 M) at 0 °C was added LAH (10 equiv), and this was stirred at rt for 20 h. The reaction was cooled to 0 °C and worked up by the Fieser method (for each gram of LAH were added water (1 mL), then 15% aqueous NaOH (1 mL), then water (3 mL)). This was warmed to rt and stirred for 15 min, then filtered through Celite, rinsing with THF, concentrated, and purified by RP-HPLC and/or FCC to provide the desired product.

General Procedure F: Tosyl Deprotection with Potassium Hydroxide. A mixture of the tosyl-protected indole (1.0 equiv), KOH (10 equiv), and either isoamylamine (5 equiv) or 28% aqueous ammonium hydroxide (20−30 equiv) in MeOH or EtOH (0.08 M)

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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was heated via microwave irradiation at 100−110 °C for 1−2 h. The reactions were worked up and purified by different methods as outlined in the individual examples to provide the desired products.

General Procedure G: Boc Deprotection with TFA. To a solution of the Boc-protected indole (1.0 equiv) in DCM (0.1 M) at 0 °C was added TFA (30 equiv), and the reaction was stirred at rt for 30 min to 2 h, then concentrated to provide the desired product which was used without further purification.

General Procedure H: Boc Deprotection with K_2CO_3. To a solution of the Boc-protected indole (1.0 equiv) in MeOH (0.04 M) was added K_2CO_3 (5−10 equiv), and this was heated at 70−80 °C (reflux) for 1.5−4 h. The reactions were worked up and purified by different methods as outlined in the individual examples to provide the desired products.

General Procedure I: One-Pot Chloro Displacement and Tosyl Deprotection. To a solution of tosyl-protected chloroindole (1.0 equiv) in EtOH (0.07 M) were added DIPEA (3.0−6.0 equiv) and the desired amine (1.5−2.0 equiv), and this was heated via microwave irradiation at 50−120 °C for 15−60 min. The reaction was cooled to rt, KOH (10 equiv) and 28% aqueous ammonium hydroxide (50 equiv) were added, and this was heated again via microwave irradiation at 80−100 °C for 1−2 h. The reaction was diluted with saturated aqueous brine, extracted 3× with DCM, dried with Na_2SO_4, filtered, concentrated, and purified by FCC or RP-HPLC to provide the desired product.

General Procedure J: Chloro Displacement Using K_2CO_3/DMSO. To a solution of the amine (1.0−2.0 equiv) in DMSO (0.15−0.4 M) was added K_2CO_3 (2.0−8.0 equiv), and this was stirred at rt for 5 min. The chloride (1.0 equiv) was added, and this was stirred at 80−90 °C for 1−15 h. The reaction was cooled to rt, diluted with water, extracted with EtOAc. The organic extract was washed with water and saturated aqueous brine, dried with Na_2SO_4, filtered, and concentrated to give the crude material, which was either used without further purification or purified by FCC to provide the desired product.

General Procedure K: Chloro Displacement Using DIPEA/DMA/EtOH. To a solution of the amine (1.0−1.5 equiv) in dimethylacetamide and/or EtOH (0.1−0.3 M) was added DIPEA (2.5−5.0 equiv) followed by the chloride (1.0 equiv), and this was stirred at 80−100 °C for 1−3 h. The reaction was cooled to rt, concentrated, and purified by FCC to provide the desired product.

General Procedure L: Nitrile Hydrolysis and Tosyl Deprotection. To a solution of the nitrile-containing tosyl-protected indole (1.0 equiv) in EtOH (0.14 M) was added KOH (8.0 equiv), and this was stirred and heated at 130 °C via microwave irradiation for 2.5 h. The reaction was cooled to rt, acidified with saturated aqueous citric acid, extracted 2× with 9:1 DCM/TFE, dried with Na_2SO_4, filtered, and concentrated to provide the desired product which was used without further purification.

General Procedure M: Esterification with TMS-diazomethane. To a solution of the carboxylic acid (1.0 equiv) in 3:1 toluene/MeOH (0.02−0.1 M) was added dropwise TMS-diazomethane (2 M in Et_2O, 2.5−9.0 equiv), and this was stirred at rt for 0.5−2 h. Acetic acid was added until gas stopped evolving, then the mixture was extracted with EtOAc, and the organic extract was washed successively with 5% aqueous sodium bicarbonate (twice), water, and saturated aqueous brine, dried with Na_2SO_4, filtered, concentrated, and purified by FCC to provide the desired product.

General Procedure N: Ester Hydrolysis. To a solution of the ester (1.0 equiv) in MeOH and/or THF (0.05−0.15 M) was added 1 M aqueous LiOH (3.0−9.0 equiv), and this was stirred either at rt for 16 h or at 70 °C for 1−8 h, as denoted in the individual examples. The reaction was concentrated and purified by RP-HPLC to provide the desired product.

General Procedure O: Reductive Amination. To a solution of the amine (1.0 equiv) and aldehyde (1.0−1.5 equiv) in DCE (0.2 M) was added $NaBH(OAc)_3$ (3.0−5.0 equiv), and this was stirred at rt for 16−40 h. The reaction was diluted with EtOAc, washed successively with 5% aqueous sodium bicarbonate, water, and saturated aqueous brine, dried with Na_2SO_4, filtered, and concentrated to give the crude material, which was either used without further purification or purified by FCC to provide the desired product.

***N*-(2-Bromo-4-methylnaphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine (1).** *Step 1: N-(4-Methylnaphthalen-1-yl)-4,5-dihydro-1H-imidazol-2-amine (**59**).* To a solution of 4-methylnaphthalen-1-amine (CAS no. 4523-45-9, 150 mg, 0.954 mmol) in isobutyl alcohol (5 mL) was added 2-imidazolinesulfonic acid (300 mg, 2 mmol). The reaction was stirred at reflux for 4 h, then cooled to rt, concentrated, and purified by RP-HPLC-A. Fractions containing product were diluted with DCM and saturated aqueous $NaHCO_3$. The layers were separated and the organic layer was dried, filtered and concentrated to provide the title compound (50 mg, 23%). MS (ESI +) m/z 226.0 (M + H).

*Step 2: N-(2-Bromo-4-methylnaphthalen-1-yl)-4,5-dihydro-1H-imidazol-2-amine (**1**).* To a solution of **59** (45 mg, 0.200 mmol) in DCM (2 mL) at 0 °C was added a solution of Br_2 (2 M in DCM, 10.3 μL, 0.200 mmol) in DCM (1 mL) dropwise. The reaction was stirred for 2 h and then diluted with 1 M aqueous NaOH, extracted with DCM, dried, filtered, concentrated, and purified by RP-HPLC-A. Fractions containing product were diluted with DCM and saturated aqueous $NaHCO_3$. The resulting layers were separated and the organic layer was dried, filtered, and concentrated to provide the title compound (21 mg, 35%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.08−7.52 (m, 5H), 3.60 (br s, 4H), 2.68 (s, 3H). HRMS calcd for $C_{14}H_{15}BrN_3$ $(M + H)^+$ 304.0449 and 306.0429, found 304.0457 and 306.0438.

***N*-(2-Chloro-4-methylnaphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine (2).** *Step 1: 2-Chloro-4-methylnaphthalen-1-amine (**60**).* General procedure A with 4-methylnaphthalen-1-amine (CAS no. 4523-45-9, 2.8 g, 17.8 mmol) and NCS, followed by FCC purification (0−20% EtOAc/heptanes), provided the title compound (1.56 g, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (dd, J = 8.1, 1.6 Hz, 1H), 7.87 (dd, J = 7.8, 1.6 Hz, 1H), 7.50 (m, 2H), 7.19 (s, 1H), 5.68 (s, 2H), 2.48 (s, 3H). MS (ESI+) m/z 192.1 (M + H).

*Step 2: 1-(2-Chloro-4-methylnaphthalen-1-yl)-3-methylthiourea (**61**).* General procedure B with **60** (1.2 g, 6.26 mmol) and methylamine (33% in ethanol, 3 mL, 24.1 mmol) provided the title compound (1.42 g, 86%) which was used without further purification. MS (ESI+) m/z 265.1 (M + H).

*Step 3: N-(2-Chloro-4-methylnaphthalen-1-yl)-4,5-dihydro-1H-imidazol-2-amine (**2**).* General procedure C with **61** (50 mg, 0.201 mmol) and ethylenediamine (0.027 mL, 0.402 mmol), followed by RP-HPLC-A, provided the title compound (15 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.34 (s, 1H), 8.13 (dd, J = 7.6, 1.8 Hz, 1H), 7.91 (dd, J = 7.4, 2.1 Hz, 1H), 7.78−7.65 (m, 2H), 7.62 (s, 1H), 3.66 (s, 4H), 2.71 (s, 3H). HRMS calcd for $C_{14}H_{15}ClN_3$ $(M + H)^+$ 260.0954, found 260.0958.

***N*-(2-Chloro-4-methylnaphthalen-1-yl)-1*H*-imidazol-2-amine (3).** *Step 1: 1-(2-Chloro-4-methylnaphthalen-1-yl)-3-(2,4,6-trimethoxybenzyl)thiourea (**62**).* General procedure B with **60** (263 mg, 1.37 mmol) and 2,4,6-trimethoxybenzylamine (641 mg, 2.74 mmol) provided the title compound (0.509 g, 86%) which was used without further purification. MS (ESI+) m/z 430.9 (M + H).

*Step 2: (E)-1-(2-Chloro-4-methylnaphthalen-1-yl)-1-(2,2-diethoxyethyl)-3-(2,4,6-trimethoxybenzyl)guanidine (**63**).* General procedure C with **62** (509 mg, 1.18 mmol) and aminoacetalaldehyde diethyl acetal (472 mg, 3.54 mmol) provided the title compound (626 mg, quant) which was used without further purification. MS (ESI+) m/z 530.3 (M + H).

*Step 3: N-(2-Chloro-4-methylnaphthalen-1-yl)-1H-imidazol-2-amine (**3**).* To a solution of **63** (626 mg, 1.18 mmol) in isopropanol (5.04 mL) was added 1.2 N aqueous HCl (15.52 mL), and the reaction was stirred at 80 °C overnight. The reaction was diluted with saturated aqueous sodium bicarbonate and extracted with DCM. The organic layers were combined and passed through an Isolute phase separator, concentrated, and purified by RP-HPLC-A. Fractions containing product were lyophilized and triturated with acetonitrile. The solid was filtered and dried to provide the title compound (4.5 mg, 1.4% for two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16−8.09 (m, 1H), 7.70−7.58 (m, 3H), 7.25−7.18 (m, 1H), 6.69−6.63

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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(m, 2H), 5.16 (s, 2H), 2.72 (d, J = 1.0 Hz, 3H). HRMS calcd for $C_{14}H_{13}ClN_3$ $(M + H)^+$ 258.0798, found 258.0858.

_N_-(2-Chloro-4-methylnaphthalen-1-yl)-1_H_-benzo[_d_]-imidazol-2-amine (4). General procedure C with **61** (150 mg, 0.567 mmol) and benzene-1,2-diamine (245 mg, 2.27 mmol) was followed except that the cyclization step was performed in DMF at 130 °C. FCC purification (0−10% MeOH/DCM), followed by RP-HPLC-A purification, provided the title compound (14 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10−7.98 (m, 2H), 7.65−7.48 (m, 3H), 7.09−7.02 (m, 2H), 6.87 (dd, J = 5.8, 3.2 Hz, 2H), 2.69 (d, J = 1.0 Hz, 3H). HRMS calcd for $C_{18}H_{15}ClN_3$ $(M + H)^+$ 308.0954, found 308.0966.

_N_-(2-Chloro-4-methylnaphthalen-1-yl)-4,5-dihydrooxazol-2-amine (5). *Step 1: 1-(2-Chloro-4-methylnaphthalen-1-yl)-3-(2-hydroxyethyl)thiourea (**64**).* General procedure B with **60** (150 mg, 0.783 mmol) and ethanolamine (0.142 mL, 2.35 mmol) provided the title compound (231 mg, quant) which was used without further purification. MS (ESI+) m/z 295.0 (M + H).

*Step 2: N-(2-Chloro-4-methylnaphthalen-1-yl)-4,5-dihydrooxazol-2-amine (**5**).* To a solution of **64** (231 mg, 0.783 mmol) in THF (8 mL) were added 2.0 M aqueous NaOH (0.98 mL, 1.96 mmol) and TsCl (164 mg, 0.861 mmol), and the reaction was stirred at rt overnight. The mixture was diluted with DCM, washed with saturated aqueous sodium bicarbonate and then saturated aqueous brine. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified by RP-HPLC-A to provide the title compound (63 mg, 31%). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ 8.01 (ddd, J = 12.8, 7.6, 2.3 Hz, 2H), 7.61−7.48 (m, 2H), 7.35 (d, J = 1.1 Hz, 1H), 5.46 (s, 1H), 4.43 (d, J = 8.1 Hz, 2H), 3.57 (t, J = 7.3 Hz, 2H), 2.64 (s, 3H). HRMS calcd for $C_{14}H_{14}ClN_2O$ $(M + H)^+$ 261.0795, found 261.0852.

_N_-(2-Chloro-4-methylnaphthalen-1-yl)-1,4,5,6-tetrahydropyrimidin-2-amine (6). General procedure C with **61** (100 mg, 0.378 mmol) and 1,3-diaminopropane (0.129 mL, 1.51 mmol) was followed except that potassium carbonate (104 mg, 0.755 mmol) was added during the cyclization step. RP-HPLC-A purification provided the title compound (5.3 mg, 5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11−8.04 (m, 1H), 7.98−7.90 (m, 1H), 7.69−7.61 (m, 2H), 7.53 (s, 1H), 3.25−3.17 (m, 4H), 2.67 (s, 3H), 1.90−1.78 (m, 2H). HRMS calcd for $C_{15}H_{17}ClN_3$ $(M + H)^+$ 274.1111, found 274.1136.

5-Bromo-_N_-(4,5-dihydro-1_H_-imidazol-2-yl)-7-methyl-1_H_-indol-4-amine (7). *Step 1: 7-Methyl-4-nitroindoline (**65**).* To 7-methylindoline (CAS no. 65673-86-1, 4.6 g, 34.5 mmol) was added sulfuric acid (135 mL), and the mixture was cooled to 0 °C (ice/salt bath). KNO_3 (3.88 g, 38.0 mmol) was added portionwise over 18 min while maintaining the internal temperature below 0 °C. The reaction was stirred in the ice/salt bath for 2.5 h, then was poured over ice. The pH of the dark purple solution was adjusted with 50% aqueous NaOH until the solution became a dark orange mixture, at which point 1 N aqueous NaOH was used to adjust the pH to neutral. The now red mixture was extracted 4× with EtOAc and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated to provide the title compound as the minor component of a ∼30:70 mixture with 7-methyl-6-nitroindoline (6.6 g total) which was used without further purification. MS (ESI+) m/z 179.2 (M + H).

*Step 2: 1-(7-Methyl-4-nitroindolin-1-yl)ethan-1-one (**66**).* The mixture of isomers containing 7-methyl-6-nitroindoline and 7-methyl-4-nitroindoline (**65**) (6.6 g, 37.0 mmol) was dissolved in THF (370 mL), and Et_3N (6.87 mL, 49.3 mmol) was added, and the reaction was cooled to 0 °C. Acetic anhydride (4.02 mL, 42.6 mmol) was added, and the reaction was stirred at rt for 1.5 h. The reaction was cooled again to 0 °C, and acetyl chloride (3.03 mL, 42.6 mmol) was added. The mixture was then partitioned between EtOAc and 1 N aqueous HCl. The organic layer was washed with water and brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound as a mixture with 1-(7-methyl-6-nitroindolin-1-yl)ethanone (7.8 g total), which was used without further purification. MS (ESI+) m/z 221.2 (M + H).

*Step 3: 1-(4-Amino-7-methylindolin-1-yl)ethan-1-one (**67**).* General procedure D with the mixture of isomers 1-(7-methyl-6-nitroindolin-1-yl)ethanone and 1-(7-methyl-4-nitroindolin-1-yl)-ethan-1-one (**66**) (7.8 g, 35.4 mmol), followed by FCC purification (0−40% EtOAc/heptanes), provided the title compound (2 g, 31% for 3 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.67 (d, J = 8.1 Hz, 1H), 6.31 (d, J = 8.0 Hz, 1H), 4.75 (s, 2H), 3.99 (t, J = 7.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.15 (s, 3H), 2.00 (s, 3H). MS (ESI+) m/z 191.3 (M + H).

*Step 4: 1-(4-Amino-5-bromo-7-methylindolin-1-yl)ethan-1-one (**68**).* General procedure A with **67** (500 mg, 2.63 mmol) and NBS, followed by FCC purification (0−50% EtOAc/heptane), provided the title compound (0.70 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.01 (s, 1H), 4.93 (s, 2H), 4.03 (t, J = 7.6 Hz, 2H), 2.82 (t, J = 7.6 Hz, 2H), 2.16 (s, 3H), 2.00 (s, 3H). MS (ESI+) m/z 271.3 (M + H).

*Step 5: 1-(1-Acetyl-5-bromo-7-methylindolin-4-yl)-3-methylthiourea (**69**).* General procedure B with **68** (700 mg, 2.60 mmol) and methylamine (33% in ethanol, 2.59 mL, 20.8 mmol), followed by FCC purification (0−5% MeOH/DCM), provided the title compound (0.85 g, 95%). MS (ESI+) m/z 344.0 (M + H).

*Step 6: 1-(5-Bromo-4-((4,5-dihydro-1H-imidazol-2-yl)amino)-7-methylindolin-1-yl)ethan-1-one (**70**).* General procedure C with **69** (0.85 g, 2.48 mmol) and ethylenediamine (0.666 mL, 9.93 mmol), followed by FCC purification (0−10% MeOH/DCM, MeOH contains 10% ammonium hydroxide), provided the title compound (0.46 g, 55%). MS (ESI+) m/z 339.0 (M + H).

*Step 7: 5-Bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-7-methyl-1H-indol-4-amine (**7**).* To a solution of **70** (100 mg, 0.297 mmol) in MeOH (3 mL) was added ∼8.4 N aqueous NaOH solution (obtained by diluting 50% NaOH solution, 0.177 mL, 1.48 mmol). The reaction was stirred at rt for 12 h, then at 50 °C for 7 h. Additional aqueous NaOH (0.177 mL, 1.48 mmol) was added, and the reaction was stirred at 50 °C for 16 h, then cooled and purified directly by FCC (0−20% MeOH/DCM, MeOH contains 10% ammonium hydroxide) to provide the title compound (14.6 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.38 (br s, 1 H), 7.36 (d, J = 2.78 Hz, 1 H), 7.12 (s, 1 H), 6.31 (d, J = 3.03 Hz, 1 H), 3.51 (s, 4 H), 2.45 (s, 3 H). HRMS calcd for $C_{12}H_{14}BrN_4$ $(M + H)^+$ 293.0402, found 293.0409.

5-Bromo-3-chloro-_N_-(4,5-dihydro-1_H_-imidazol-2-yl)-7-methyl-1_H_-indol-4-amine (8). General procedure A with **7** (70 mg, 0.239 mmol) and NCS, followed by FCC purification (0−20% MeOH/DCM, MeOH contains 10% ammonium hydroxide), provided the title compound (16 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (d, J = 2.8 Hz, 1H), 10.32 (s, 1H), 8.31 (s, 1H), 7.98 (s, 1H), 7.66 (d, J = 2.8 Hz, 1H), 7.32 (s, 1H), 3.71 (m, 2H), 3.56 (m, 2H), 2.48 (s, 3H). HRMS calcd for $C_{12}H_{13}BrClN_4$ $(M + H)^+$ 327.0012, found 327.0013.

_N_-(4,5-Dihydro-1_H_-imidazol-2-yl)-5,7-dimethyl-1_H_-indol-4-amine (9). *Step 1: 1-(5,7-Dimethyl-1H-indol-4-yl)-3-methylthiourea (**71**).* General procedure B with 5,7-dimethyl-1_H_-indol-4-amine (**93**; see Supporting Information) (202 mg, 1.26 mmol) and methylamine (33% in ethanol, 0.261 mL, 6.31 mmol), followed by FCC purification (0−20% EtOAc/DCM), provided the title compound (294 mg, quant). MS (ESI+) m/z 234.4 (M + H).

*Step 2: N-(4,5-Dihydro-1H-imidazol-2-yl)-5,7-dimethyl-1H-indol-4-amine (**9**).* General procedure C with **71** (100 mg, 0.429 mmol) and ethylenediamine (0.058 mL, 0.857 mmol), followed by FCC purification [0−60% (2 M NH_3 in MeOH)/EtOAc] and RP-HPLC-A purification, provided the title compound (40 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.77 (br s, 1 H), 7.14 (d, J = 2.53 Hz, 1 H), 6.67 (s, 1 H), 6.17 (d, J = 2.53 Hz, 1 H), 3.35 (br s, 2 H), 3.31 (br s, 2 H), 2.37 (s, 3 H), 2.11 (s, 3 H). HRMS calcd for $C_{13}H_{17}N_4$ $(M + H)^+$ 229.1453, found 229.1452.

_N_-(4,5-Dihydro-1_H_-imidazol-2-yl)-7-methyl-1_H_-indol-4-amine (11). *Step 1: 1-Methyl-3-(7-methyl-1-tosyl-1H-indol-4-yl)-thiourea (**72**).* General procedure B with 7-methyl-1-tosyl-1_H_-indol-4-amine (**95**; see Supporting Information) (300 mg, 0.999 mmol) and methylamine (33% in ethanol, 0.207 mL, 4.99 mmol), followed by FCC purification [40−100% (30% EtOAc in DCM)/heptane], provided the title compound (310 mg, 83%). MS (ESI+) m/z 374.2 (M + H).

*Step 2: N-(4,5-Dihydro-1H-imidazol-2-yl)-7-methyl-1-tosyl-1H-indol-4-amine (**73**).* General procedure C with **72** (310 mg, 0.830

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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mmol) and ethylenediamine (0.280 mL, 4.15 mmol), followed by FCC purification [EtOAc wash, then 0−70% (2 M NH_3 in MeOH)/DCM] and trituration with EtOH, provided the title compound (270 mg, 88%). MS (ESI+) m/z 369.1 (M + H).

*Step 3: N-(4,5-Dihydro-1H-imidazol-2-yl)-7-methyl-1H-indol-4-amine (**11**).* To a solution of **73** (70 mg, 0.190 mmol) in EtOH (2 mL) was added NaOEt (21% in EtOH, 0.074 mL, 0.950 mmol) and THF (1 mL), and this was stirred at 80 °C for 15 h. The mixture was cooled to rt, neutralized with AcOH, and directly purified by RP-HPLC-A to provide the title compound as the TFA salt (30 mg, 42%). ^{1}H NMR (400 MHz, DMSO-d_6, TFA salt) δ ppm 11.37 (br s, 1 H), 10.34 (s, 1 H), 8.04 (s, 2 H), 7.45−7.39 (m, 1 H), 6.94 (d, J = 8.08 Hz, 1 H), 6.83 (d, J = 8.08 Hz, 1 H), 6.45−6.39 (m, 1 H), 3.63 (s, 4 H), 2.48 (s, 3 H). HRMS calcd for $C_{12}H_{15}N_4$ $(M + H)^+$ 215.1288, found 215.1291.

N-(4,5-Dihydro-1H-imidazol-2-yl)-5-methyl-1H-indol-4-amine (12). To a mixture of 5-methyl-1-tosyl-1*H*-indol-4-amine (**98**; see Supporting Information) (43.6 mg, 0.145 mmol) and 1-acetylimidazolidin-2-one (20.5 mg, 0.160 mmol) under nitrogen was added $POCl_3$ (0.97 mL, 10.4 mmol), and this was stirred at 50 °C overnight. The mixture was concentrated, sodium ethoxide (21% in ethanol, 110 μL, 0.29 mmol) and ethanol (0.6 mL) were added, and the reaction was stirred at rt. After 2 h additional sodium ethoxide (21% in ethanol, 542 μL, 1.45 mmol) was added and the reaction was stirred at 50 °C for 1 h, then additional sodium ethoxide (21% in ethanol, 542 μL, 1.45 mmol) was added and the reaction was stirred at 80 °C for 2 h. The reaction was filtered, rinsing with MeOH, and purified by RP-HPLC-A to provide the title compound as the TFA salt (14 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.27 (br s, 1 H), 10.11 (s, 1 H), 7.98 (br s, 2 H), 7.43−7.31 (m, 2 H), 7.04 (d, J = 8.34 Hz, 1 H), 6.28 (t, J = 2.15 Hz, 1 H), 3.63 (s, 4 H), 2.27 (s, 3 H). HRMS calcd for $C_{12}H_{15}N_4$ $(M + H)^+$ 215.1290, found 215.1291.

4-((4,5-Dihydro-1H-imidazol-2-yl)methyl)-5,7-dimethyl-1H-indole (13). *Step 1: 4-(2,2-Dibromovinyl)-5,7-dimethyl-1-tosyl-1H-indole (**44**).* To a solution of **43** (100 mg, 0.305 mmol) and CBr_4 (152 mg, 0.458 mmol) in DCM (3 mL) at 0 °C was added PPh_3 (240 mg, 0.916 mmol), and the mixture was stirred at 0 °C for 3.5 h, then purified directly by FCC [0−50% (30% EtOAc in DCM)/heptane] to provide the title compound (125 mg, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.85 (s, 1H), 7.83 (d, J = 3.79 Hz, 1H), 7.58 (d, J = 8.34 Hz, 2H), 7.42−7.37 (m, 2H), 6.95 (s, 1H), 6.63 (d, J = 3.79, 1H), 2.44 (s, 3H), 2.33 (s, 3H), 2.19 (s, 3H).

*Step 2: 4-((4,5-Dihydro-1H-imidazol-2-yl)methyl)-5,7-dimethyl-1H-indole (**13**).* A solution of **44** (125 mg, 0.259 mmol) in ethylenediamine (1 mL) was stirred at rt for 9 h. The mixture was diluted with DCM, washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated. This was dissolved in EtOH (2 mL), and isoamylamine (22.5 mg, 0.259 mmol) and sodium ethoxide (21% in EtOH, 0.5 mL) were added, and the reaction was heated at 85 °C overnight. The reaction was cooled to rt, neutralized with AcOH, and purified directly by RP-HPLC-A to provide the title compound as the TFA salt (45 mg, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.12 (br s, 1H), 9.60 (s, 1H), 7.38−7.32 (m, 1H), 6.79 (s, 1H), 6.46 (dd, J = 3.03, 1.77 Hz, 1H), 4.06 (s, 2H), 3.80−3.74 (m, 4H), 2.43 (s, 3H), 2.30 (s, 3H). HRMS calcd for $C_{14}H_{18}N_3$ $(M + H)^+$ 228.1501, found 228.1496.

4-((1H-1,2,4-Triazol-5-yl)methyl)-5,7-dimethyl-1H-indole (14). *Step 1: (5,7-Dimethyl-1-tosyl-1H-indol-4-yl)(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-1,2,4-triazol-5-yl)methanol (**45**).* To a solution of 1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-1,2,4-triazole (CAS no. 288-88-0, 82 mg, 0.412 mmol) in THF (2 mL) at −78 °C was added *n*-BuLi (1.6 M in hexanes, 0.241 mL, 0.385 mmol), and this was stirred for 1 h, then added to a solution of **43** (90 mg, 0.275 mmol) in THF (2 mL) at −78 °C. This was stirred at −78 °C for 1 h, then diluted with half saturated aqueous $KHSO_4$ and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to provide the title compound (130 mg, 90%) which was used without further purification. MS (ESI+) m/z 527.0 (M + H).

*Step 2: 4-((1H-1,2,4-Triazol-5-yl)methyl)-5,7-dimethyl-1H-indole (**14**).* To a solution of **45** (130 mg, 0.247 mmol) and triethylamine (0.103 mL, 0.740 mmol) in DCM (5 mL) at 0 °C was added MsCl (0.038 mL, 0.494 mmol), and this was stirred at 0 °C for 5 h. The mixture was diluted with EtOAc, washed with H_2O and brine, dried with Na_2SO_4, filtered, and concentrated. The resulting residue was dissolved in THF (10 mL) and cooled to 0 °C. $LiAlH_4$ (94 mg, 2.47 mmol) was added, and the reaction was stirred at rt overnight. The mixture was cooled to 0 °C, and H_2O (100 μL), 15% aqueous NaOH (100 μL), and H_2O (300 μL) were added carefully. The mixture was stirred at rt for 1 h, then Celite was added and the solid was filtered off through Celite. The filtrate was concentrated and purified by FCC to provide a mixture of intermediates 5,7-dimethyl-1-tosyl-4-((1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-1,2,4-triazol-5-yl)methyl)-1*H*-indole (MS (ESI+) m/z 511.0 (M + H)) and 5,7-dimethyl-4-((1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-1,2,4-triazol-5-yl)methyl)-1*H*-indole (MS (ESI+) m/z 357.0 (M + H)). This mixture was dissolved in THF (1 mL), and TBAF trihydrate (278 mg, 0.881 mmol) was added. The reaction was stirred at rt for 1.5 h, then at 45 °C for 6 h. The reaction was cooled to rt, additional TBAF trihydrate (278 mg, 0.881 mmol) was added, and this was stirred at 45 °C for 9 h, then cooled to rt, diluted with H_2O, and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, concentrated, and purified by RP-HPLC-A. Fractions containing product were basified with saturated aqueous sodium bicarbonate, extracted with DCM, partitioned by an Isolute phase separator, and concentrated to provide the title compound (13 mg, 23% for 3 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.54 (br s, 1H), 10.91 (br s, 1H), 7.93 (br s, 1H), 7.28−7.15 (m, 1H), 6.69 (s, 1H), 6.48 (dd, J = 3.03, 2.02 Hz, 1H), 4.19 (s, 2H), 2.38 (s, 3H), 2.38 (s, 3H). HRMS calcd for $C_{14}H_{15}N_3$ $(M + H)^+$ 227.1291, found 227.1292.

2-((5,7-Dimethyl-1H-indol-4-yl)methyl)-1H-benzo[d]-imidazole (15). *Step 1: 2-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)-methyl)-1H-benzo[d]imidazole (**74**).* A mixture of **44** (90 mg, 0.186 mmol), 1,2-phenylenediamine (30.2 mg, 0.279 mmol), and DABCO (46.0 mg, 0.410 mmol) in NMP (2 mL) was stirred at 100 °C for 22.5 h. The reaction was cooled to rt and purified directly by FCC (0−67% EtOAc/heptanes) to provide the title compound (65 mg, 81%). MS (ESI+) m/z 430.1 (M + H).

*Step 2: 2-((5,7-Dimethyl-1H-indol-4-yl)methyl)-1H-benzo[d]-imidazole (**15**).* General procedure E was performed on **74** (65 mg, 0.151 mmol), followed by RP-HPLC-A purification. Fractions containing product were basified with saturated aqueous sodium bicarbonate, extracted with DCM, partitioned by an Isolute phase separator, and concentrated to provide the title compound (35 mg, 84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.82 (br s, 1H), 10.91 (br s, 1H), 7.45 (br s, 1H), 7.34 (br s, 1H), 7.26−7.17 (m, 1H), 7.11−6.96 (m, 2H), 6.74 (s, 1H), 6.49 (dd, J = 3.03, 1.77 Hz, 1H), 4.35 (s, 2H), 2.41 (s, 3H), 2.38 (s, 3H). HRMS calcd for $C_{14}H_{15}N_3$ $(M + H)^+$ 276.1501, found 276.1499.

2-(5,7-Dimethyl-1H-indol-4-yl)ethan-1-amine (16). *Step 1: tert-Butyl (2-(5,7-Dimethyl-1-tosyl-1H-indol-4-yl)ethyl)carbamate (**75**).* A suspension of 4-iodo-5,7-dimethyl-1-tosyl-1*H*-indole (**100**; see Supporting Information) (200 mg, 0.470 mmol), potassium (2-((*tert*-butoxycarbonyl)amino)ethyl)trifluoroborate (CAS no. 926280-83-3, 381 mg, 1.41 mmol), Cs_2CO_3 (613 mg, 1.88 mmol), and $PdCl_2$(dppf).DCM adduct (77 mg, 0.094 mmol) in toluene (3 mL) and H_2O (1 mL) was stirred at 80 °C under nitrogen overnight. The reaction was cooled to rt and purified directly by FCC [0−67% (30% EtOAc in DCM)/heptane] to afford the title compound (120 mg, 58%). ^{1}H NMR (400 MHz, dichloromethane-d_2) δ ppm 7.78 (d, J = 3.79 Hz, 1H), 7.58 (d, J = 8.34 Hz, 2H), 7.29 (d, J = 8.34 Hz, 2H), 6.89 (s, 1H), 6.86 (d, J = 3.79 Hz, 1H), 3.29 (t, J = 7.33 Hz, 2H), 3.03 (t, J = 7.33 Hz, 2H), 2.51 (s, 3H), 2.41 (s, 3H), 2.36 (s, 3H), 1.47 (s, 9H).

*Step 2: 2-(5,7-Dimethyl-1H-indol-4-yl)ethan-1-amine (**16**).* General procedure G was performed on **75** (60 mg, 0.136 mmol). The resulting residue was dissolved in EtOH (2 mL), and NaOEt (21% in EtOH, 1 mL) and isoamylamine (500 μL, 4.30 mmol) were added. This was heated at 80 °C for 20 h, then cooled to rt, acidified with AcOH, and purified by RP-HPLC-A to provide the title compound (6 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.86 (br s, 1H),

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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7.28−7.19 (m, 1H), 6.68 (s, 1H), 6.44 (dd, J = 3.03, 1.77 Hz, 1H), 4.43 (br s, 2H), 2.98−2.90 (m, 2H), 2.80−2.70 (m, 2H), 2.38 (s, 3H), 2.29 (s, 3H). HRMS calcd for $C_{12}H_{17}N_2$ $(M + H)^+$ 189.1392, found 189.1383.

4-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)morpholine (17). General procedure I with **46** (50 mg, 0.144 mmol) and morpholine (0.025 mL, 0.287 mmol), followed by RP-HPLC-B purification, provided the title compound (21 mg, 60%). ^{1}H NMR (400 MHz, dichloromethane-d_2) δ ppm 8.10 (br s, 1 H), 7.20 (br s, 1 H), 6.82 (s, 1 H), 6.65 (br s, 1 H), 3.70 (s, 2 H), 3.65−3.44 (m, 4 H), 2.45 (m, 4H), 2.44 (s, 3H), 2.43 (s, 3H). HRMS calcd for $C_{15}H_{21}N_2O$ $(M + H)^+$ 245.1648, found 245.1657.

5,7-Dimethyl-4-(piperidin-1-ylmethyl)-1*H*-indole (18). To a solution of **43** (50 mg, 0.153 mmol) and AcOH (0.017 mL, 0.305 mmol) in DCM (2 mL) and MeOH (0.5 mL) was added piperidine (0.015 mL, 0.153 mmol). The mixture was stirred for 15 min, then $NaBH(OAc)_3$ (64.7 mg, 0.305 mmol) was added, and the mixture was stirred at rt for 19 h. The reaction was diluted with DCM, washed with 5% aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, concentrated, and purified by FCC [5−15% (2 M NH_3 in MeOH)/DCM] to provide intermediate 5,7-dimethyl-4-(piperidin-1-ylmethyl)-1-tosyl-1*H*-indole (MS (ESI+) m/z 397.3 $(M + H)$). General procedure E was then performed, followed by RP-HPLC-A purification. Fractions containing product were basified with saturated aqueous sodium bicarbonate, extracted with DCM, partitioned by an Isolute phase separator, and concentrated to provide the title compound (8 mg, 22% for 2 steps). ^{1}H NMR (400 MHz, dichloromethane-d_2) δ ppm 8.03 (br s, 1H), 7.10 (t, J = 2.78 Hz, 1H), 6.73 (s, 1H), 6.56 (dd, J = 3.16, 2.15 Hz, 1H), 3.59 (br s, 2H), 2.35 (s, 3H), 2.34 (s, 3H), 2.34 (br s, 4H), 1.45 (br s, 4H), 1.33 (br s, 2H). MS (ESI+) m/z 243.2 $(M + H)$.

(*R*)-5,7-Dimethyl-4-((2-phenylpiperidin-1-yl)methyl)-1*H*-indole ((*R*)-19) and (*S*)-5,7-Dimethyl-4-((2-phenylpiperidin-1-yl)methyl)-1*H*-indole ((*S*)-19). General procedure I with **46** (50 mg, 0.144 mmol) and 2-phenylpiperidine (CAS no. 3466-80-6, 46.4 mg, 0.287 mmol), followed by FCC purification (0−60% EtOAc/heptane), gave 33 mg of (±)-**19**. The enantiomers were separated by chiral HPLC using a CHIRALPAK OD column (Waters prep-100, 20 mm × 250 mm, 10−40% (IPA + 0.05% diethylamine) in heptane) to provide the first eluting enantiomer (*R*)-**19** (t_r = 7.9 min, 7.1 mg, 16%) and the second eluting enantiomer (*S*)-**19** (t_r = 10.8 min, 6.9 mg, 15%). The absolute stereochemistry was determined via cocrystallization of (*S*)-**19** with the catalytic domain of FB. Data for (±)-**19**: ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 8.04 (br s, 1 H), 7.53 (d, J = 7.1 Hz, 2 H), 7.36 (app t, J = 7.3 Hz, 2 H), 7.26 (app t, J = 7.2 Hz, 1 H), 7.16 (br s, 1 H), 6.74 (s, 1 H), 6.68 (br s, 1 H), 3.72 (d, J = 12.4 Hz, 1 H), 3.15 (d, J = 12.4 Hz, 1 H), 3.10−3.00 (m, 1 H), 2.72 (d, J = 10.9 Hz, 1 H), 2.40 (s, 3 H), 2.31 (s, 3 H), 1.94 (t, J = 11.4 Hz, 1 H), 1.79−1.65 (m, 3 H), 1.47−1.26 (m, 3 H). HRMS calcd for $C_{22}H_{27}N_2$ $(M + H)^+$ 319.2174, found 319.2177.

(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)-methanol (20-entA) and (20-entB). General procedure I with **46** (60 mg, 0.172 mmol) and 2-piperidin-2-ylmethanol (CAS no. 3433-37-2, 39.7 mg, 0.345 mmol), followed by FCC purification (0−100% EtOAc/heptane), gave 26 mg of (±)-**20**. The enantiomers were separated by chiral HPLC using a CHIRALPAK OD column (Waters prep-100, 20 mm × 250 mm, 20% (EtOH + 0.05% diethylamine) in heptane) to provide the first eluting enantiomer **20**-ent A (t_r = 10.7 min, 4 mg, 8%) and the second eluting enantiomer **20**-ent B (t_r = 12.1 min, 5 mg, 11%). **20**-entA: ^{1}H NMR (400 MHz, methylene chloride-d_2) δ 8.17 (s, 1H), 7.22 (d, J = 3.3 Hz, 1H), 6.84 (s, 1H), 6.68 (s, 1H), 4.32 (s, 1H), 3.98−3.37 (m, 3H), 2.83 (s, 1H), 2.65−2.21 (m, 8H), 1.90−1.42 (m, 7H). HRMS calcd for $C_{17}H_{25}N_2O$ $(M + H)^+$ 273.1967, found 273.1969. **20**-entB: ^{1}H NMR (400 MHz, methylene chloride-d_2) δ 8.17 (s, 1H), 7.22 (d, J = 3.3 Hz, 1H), 6.84 (s, 1H), 6.68 (s, 1H), 4.32 (s, 1H), 3.98−3.37 (m, 3H), 2.83 (s, 1H), 2.65−2.21 (m, 8H), 1.90−1.42 (m, 7H). HRMS calcd for $C_{17}H_{25}N_2O$ $(M + H)^+$ 273.1967, found 273.1969.

(±)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1*H*-indole ((±)-21 (trans)) and (±)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1*H*-indole ((±)-22 (cis)). *Step 1: (±)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1-tosyl-1H-indole ((±)-**76**, Diastereomeric Mixture).* General procedure J with (±)-4-methoxy-2-phenylpiperidine ((±)-**113**; see Supporting Information) (180 mg, 0.789 mmol) and **46** (200 mg, 0.575 mmol) provided the title compound (206 mg, 71%) as a diastereomeric mixture which was used without further purification. MS (ESI+) m/z 503.5 $(M + H)$.

*Step 2: (±)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1H-indole ((±)-**21** (trans)) and (±)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1H-indole ((±)-**22** (cis)).* General procedure F was performed on (±)-**76** (206 mg, 0.409 mmol). The reaction mixture was diluted with DCM, filtered through a silica plug, rinsing with 6:1 DCM/MeOH, and concentrated. Separation of the diastereomers was achieved by FCC [14% (10% MeOH in EtOAc)/DCM] with mixed fractions repurified by FCC [23% (10% MeOH in EtOAc)/heptane] and combined to provide the first eluting product (±)-**22** (cis) (50 mg, 34%) and the second eluting product (±)-**21** (trans) (35 mg, 24%). (±)-**22** (cis): ^{1}H NMR (400 MHz, CD_3CN) δ 9.09 (br s, 1H), 7.54 (d, J = 7.20 Hz, 2H), 7.38 (dd, J = 7.20, 7.80 Hz, 2H), 7.31−7.26 (m, 1H), 7.16 (dd, J = 2.80, 3.00 Hz, 1H), 6.71 (s, 1H), 6.57−6.54 (m, 1H), 3.62 (d, J = 12.13 Hz, 1H), 3.29−3.19 (m, 4H), 3.18−3.10 (m, 2H), 2.64 (td, J = 3.54, 11.87 Hz, 1H), 2.38 (s, 3H), 2.25 (s, 3H), 2.10−2.02 (m, 1H), 2.02−1.97 (m, 1H), 1.90−1.79 (m, 1H), 1.55 (dd, J = 11.40, 12.13 Hz, 1H), 1.25−1.14 (m, 1H). HRMS calcd for $C_{23}H_{29}N_2O$ $(M + H)^+$ 349.2280, found 349.2278. (±)-**21** (trans): ^{1}H NMR (400 MHz, CD_3CN) δ 9.08 (br s, 1H), 7.54 (d, J = 7.33 Hz, 2H), 7.37 (dd, J = 7.33, 7.80 Hz, 2H), 7.30−7.25 (m, 1H), 7.17−7.14 (m, 1H), 6.70 (s, 1H), 6.58 (dd, J = 2.02, 3.03 Hz, 1H), 3.63 (d, J = 12.13 Hz, 1H), 3.50−3.45 (m, 1H), 3.41 (dd, J = 3.41, 11.24 Hz, 1H), 3.27 (s, 3H), 3.19 (d, J = 12.13 Hz, 1H), 2.38 (s, 3H), 2.37−2.32 (m, 1H), 2.31−2.21 (m, 4H), 1.91−1.78 (m, 2H), 1.77−1.70 (m, 1H), 1.54−1.45 (m, 1H). HRMS calcd for $C_{23}H_{29}N_2O$ $(M + H)^+$ 349.2280, found 349.2276.

(+)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1*H*-indole ((+)-21) and (−)-4-((4-Methoxy-2-phenylpiperidin-1-yl)methyl)-5,7-dimethyl-1*H*-indole ((−)-21). Resolution of the enantiomers of (±)-**21** (trans) (21 mg, 0.060 mmol) was achieved by chiral SFC using a CHIRALPAK IB column with 30% (10 mM NH_4OH in ^{i}PrOH) in CO_2 to provide (+)-**21** (t_r = 3.1 min, 8 mg, 37%) $[\alpha]_{25}^{D}$ +67.2° (c 0.1, MeOH) and (−)-**21** (t_r = 4.3 min, 9 mg, 42%) $[\alpha]_{25}^{D}$ −67.2° (c 0.1, MeOH).

(±)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-2-phenylpiperidin-4-ol ((±)-23 (trans)) and (±)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-2-phenylpiperidin-4-ol ((±)-24 (cis)). *Step 1: (±)-1-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-ol ((±)-**77**, Diastereomeric Mixture).* General procedure J with (±)-2-phenylpiperidin-4-ol ((±)-**114**; see Supporting Information) (154 mg, 0.72 mmol) and **46** (170 mg, 0.489 mmol) followed by FCC purification (25% EtOAc/heptane) provided the title compound (200 mg, 84%) as a diastereomeric mixture which was used without further purification. MS (ESI+) m/z 489.4 $(M + H)$.

*Step 2: (±)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-ol ((±)-**23** (trans)) and (±)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-ol ((±)-**24** (cis)).* General procedure F was performed on (±)-77 (200 mg, 0.409 mmol). The reaction mixture was diluted with DCM, filtered through a silica plug, rinsing with 6:1 DCM/MeOH, and concentrated. Separation of the diastereomers was achieved by FCC (7−15% MeOH/DCM) to provide the first eluting product (±)-**24** (cis) (50 mg, 36%) and the second eluting product (±)-**23** (trans) (37 mg, 26%). (±)-**24** (cis): ^{1}H NMR (400 MHz, CD_3CN) δ 9.12 (br s, 1H), 7.53 (d, J = 7.33 Hz, 2H), 7.38 (dd, J = 7.33, 7.80 Hz, 2H), 7.32−7.26 (m, 1H), 7.16 (dd, J = 2.80, 3.03 Hz, 1H), 6.71 (s, 1H), 6.56 (dd, J = 2.02, 3.03 Hz, 1H), 3.63 (d, J = 12.13 Hz, 1H), 3.60−3.53 (m, 1H), 3.19−3.14 (m, 1H), 3.12 (d, J = 12.13 Hz, 1H), 2.80 (br s, 1H), 2.65−2.59 (m, 1H), 2.38 (s, 3H), 2.25 (s, 3H), 2.05−1.96 (m, 1H), 1.91−1.87 (m, 1H), 1.75−1.68 (m, 1H), 1.67−1.56 (m, 1H), 1.34−1.21 (m, 1H). HRMS calcd for $C_{22}H_{27}N_2O$ $(M + H)^+$ 335.2123, found 335.2119. (±)-**23** (trans): ^{1}H NMR (400 MHz, CD_3CN) δ 9.09 (br s, 1H), 7.54 (d, J = 7.30 Hz,

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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2H), 7.37 (dd, J = 7.30, 7.80 Hz, 2H), 7.32−7.23 (m, 1H), 7.21−7.12 (m, 1H), 6.71 (s, 1H), 6.63−6.55 (m, 1H), 4.00−3.91 (m, 1H), 3.66 (d, J = 12.13 Hz, 1H), 3.53 (br d, J = 8.80 Hz, 1H), 3.23 (br d, J = 10.90 Hz, 1H), 2.64 (br s, 1H), 2.48−2.31 (m, 5H), 2.27 (s, 3H), 1.91−1.84 (m, 1H), 1.78−1.68 (m, 1H), 1.66−1.43 (m, 2H). HRMS calcd for $C_{22}H_{27}N_2O$ $(M + H)^+$ 335.2123, found 335.2123.

(±)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-*N*-methyl-2-phenylpiperidin-4-amine ((±)-25 (trans)). *Step 1: (±)-Benzyl (1-((5,7-dimethyl-1-tosyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-yl)carbamate ((±)-**78** (trans)).* General procedure J with (±)-benzyl (2-phenylpiperidin-4-yl)carbamate ((±)-**120** (trans); see Supporting Information) (178 mg, 0.512 mmol) and **46** (150 mg, 0.431 mmol) provided the title compound (260 mg, 97%) which was used without further purification. MS (ESI+) m/z 622.6 (M + H).

*Step 2: (±)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-N-methyl-2-phenylpiperidin-4-amine ((±)-**25** (trans)).* Starting with (±)-**78** (trans) (100 mg, 0.161 mmol), general procedure E was followed except that the reaction was heated at 50 °C for 2.5 h. RP-HPLC-A purification followed by additional FCC purification [10% (2 M NH_3 in MeOH)/DCM] provided the title compound (22 mg, 37%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (br d, J = 7.10 Hz, 2H), 7.37 (dd, J = 7.30, 8.10 Hz, 2H), 7.32−7.26 (m, 1H), 7.14 (d, J = 3.15 Hz, 1H), 6.67 (s, 1H), 6.51 (d, J = 3.15 Hz, 1H), 3.71 (d, J = 12.13 Hz, 1H), 3.20−3.11 (m, 2H), 2.85 (td, J = 3.35, 12.00 Hz, 1H), 2.60−2.50 (m, 1H), 2.40 (s, 3H), 2.34 (s, 3H), 2.24 (s, 3H), 2.11−2.03 (m, 1H), 2.03−1.96 (m, 1H), 1.85−1.77 (m, 1H), 1.63−1.53 (m, 1H), 1.35−1.23 (m, 1H). HRMS calcd for $C_{23}H_{30}N_3$ $(M + H)^+$ 348.2440, found 348.2430.

(±)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-*N*-methyl-2-phenylpiperidin-4-amine ((±)-26 (cis)). *Step 1: (±)-Benzyl (1-((5,7-dimethyl-1-tosyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-yl)carbamate ((±)-**79** (cis)).* General procedure J with (±)-benzyl (2-phenylpiperidin-4-yl)carbamate ((±)-**121** (cis); see Supporting Information) (101 mg, 0.29 mmol) and **46** (100 mg, 0.29 mmol) provided the title compound (100 mg, 56%) which was used without further purification. MS (ESI+) m/z 622.6 (M + H).

*Step 2: (±)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-N-methyl-2-phenylpiperidin-4-amine ((±)-**26** (cis)).* Starting with (±)-**79** (cis) (100 mg, 0.161 mmol), general procedure E was followed except that the reaction was heated at 50 °C for 15 h. RP-HPLC-A purification followed by FCC purification [10% (2 M NH_3 in MeOH)/DCM] provided the title compound (11 mg, 18%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (d, J = 7.30 Hz, 2H), 7.36 (dd, J = 7.30, 7.80 Hz, 2H), 7.30−7.25 (m, 1H), 7.14 (d, J = 3.30 Hz, 1H), 6.68 (s, 1H), 6.54 (d, J = 3.28 Hz, 1H), 3.75 (d, J = 12.13 Hz, 1H), 3.50 (dd, J = 3.16, 11.49 Hz, 1H), 3.29 (br d, J = 12.10 Hz, 1H), 2.83−2.79 (m, 1H), 2.65−2.58 (m, 1H), 2.47−2.42 (m, 1H), 2.39 (br s, 6H), 2.25 (s, 3H), 2.06−1.96 (m, 1H), 1.90−1.83 (m, 1H), 1.77−1.68 (m, 2H). HRMS calcd for $C_{23}H_{30}N_3$ $(M + H)^+$ 348.2440, found 348.2426.

(±)-(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-2-phenyl-piperidin-4-yl)methanol ((±)-27 (trans)). *Step 1: (±)-(1-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-yl)-methanol ((±)-**80** (trans)).* General procedure J with (±)-(2-phenylpiperidin-4-yl)methanol ((±)-**125** (trans); see Supporting Information) (100 mg, 0.44 mmol) and **46** (100 mg, 0.29 mmol) followed by FCC purification [55−95% (30% EtOAc in DCM)/heptane] provided the title compound (40 mg, 18%). MS (ESI+) m/z 503.5 (M + H).

*Step 2: (±)-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-2-phenyl-piperidin-4-yl)methanol ((±)-**27** (trans)).* General procedure F was performed on (±)-**80** (trans) (77 mg, 0.153 mmol). The reaction mixture was diluted with DCM, filtered through a silica plug, rinsing with 6:1 DCM/MeOH, and concentrated. RP-HPLC-A purification provided the title compound as the TFA salt (13 mg, 14%). ^{1}H NMR (TFA salt, 400 MHz, D_2O) δ 7.63−7.49 (m, 5H), 7.26 (d, J = 3.03 Hz, 1H), 6.78 (s, 1H), 6.12 (br s, 1H), 4.46 (dd, J = 2.65, 13.26 Hz, 1H), 4.20 (d, J = 13.40 Hz, 1H), 4.10 (d, J = 13.40 Hz, 1H), 3.77 (d, J = 7.83 Hz, 2H), 3.29−3.15 (m, 2H), 2.40−2.26 (m, 4H), 2.14−1.95 (m, 5H), 1.90−1.70 (m, 2H). HRMS calcd for $C_{23}H_{29}N_2O$ $(M + H)^+$ 349.2280, found 349.2270.

(±)-(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-2-phenyl-piperidin-4-yl)methanol ((±)-28 (cis)). *Step 1: (±)-(1-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)methyl)-2-phenylpiperidin-4-yl)-methanol ((±)-**81** (cis)).* General procedure J with (±)-(2-phenylpiperidin-4-yl)methanol ((±)-**126** (cis); see Supporting Information) (155 mg, 0.68 mmol) and **46** (200 mg, 0.575 mmol) followed by FCC purification (32−43% EtOAc/heptane) provided the title compound (77 mg, 23%). MS (ESI+) m/z 503.5 (M + H).

*Step 2: (±)-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-2-phenyl-piperidin-4-yl)methanol ((±)-**28** (cis)).* General procedure F was performed on (±)-**81** (cis) (77 mg, 0.153 mmol). The reaction mixture was diluted with DCM, filtered through a silica plug, rinsing with 6:1 DCM/MeOH, and concentrated. RP-HPLC-A purification provided the title compound as the TFA salt (65 mg, 75%). ^{1}H NMR (TFA salt, 400 MHz, D_2O) δ 7.67−7.47 (m, 5H), 7.25 (d, J = 3.03 Hz, 1H), 6.78 (s, 1H), 6.12 (br s, 1H), 4.40 (br dd, J = 2.90, 12.30 Hz, 1H), 4.23 (d, J = 13.60 Hz, 1H), 4.07 (d, J = 13.60 Hz, 1H), 3.39 (d, J = 6.32 Hz, 2H), 3.38−3.33 (m, 1H), 3.29−3.18 (m, 1H), 2.32 (s, 3H), 2.13−2.05 (m, 1H), 2.03−1.91 (m, 4H), 1.89−1.77 (m, 2H), 1.38−1.24 (m, 1H). HRMS calcd for $C_{23}H_{29}N_2O$ $(M + H)^+$ 349.2280, found 349.2265.

((2*S*,4*S*)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-4-methoxy-piperidin-2-yl)methanol (29). General procedure I was performed with **46** (50 mg, 0.144 mmol) and ((2*S*,4*S*)-4-methoxypiperidin-2-yl)methanol hydrochloride (prepared as described in ref 13, 39.4 mg, 0.216 mmol), followed by RP-HPLC-A purification. Fractions containing product were basified with saturated aqueous $NaHCO_3$, extracted with DCM, dried with Na_2SO_4, filtered, and concentrated to provide the title compound (13 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.80 (br s, 1 H), 7.21 (t, J = 2.7 Hz, 1 H), 6.67 (s, 1 H), 6.44−6.57 (m, 1 H), 4.34 (t, J = 5.2 Hz, 1 H), 4.14 (d, J = 12.6 Hz, 1 H), 3.68 (ddd, J = 10.5, 5.2, 5.1 Hz, 1 H), 3.55 (d, J = 12.4 Hz, 1 H), 3.48 (ddd, J = 11.1, 5.9, 5.7 Hz, 1 H), 3.44−3.35 (m, 1 H), 3.20 (s, 3 H), 2.47−2.21 (m, 9 H), 1.66 (t, J = 5.2 Hz, 2 H), 1.52−1.32 (m, 2 H). HRMS calcd for $C_{18}H_{27}N_2O_2$ $(M + H)^+$ 303.2073, found 303.2075.

(±)-*N*-((1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)methyl)methanesulfonamide (30). *Step 1: (±)-tert-Butyl ((1-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)methyl)piperidin-2-yl)methyl)-carbamate (**82**).* General procedure K with *tert*-butyl piperidin-2-ylmethylcarbamate (CAS no. 141771-61-0, 444 mg, 2.07 mmol) and **46** (600 mg, 1.73 mmol) followed by FCC purification (26−47% EtOAc/heptane) provided the title compound (840 mg, 93%). MS (ESI+) m/z 526.4 (M + H).

*Step 2: (±)-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)piperidin-2-yl)methanamine (**83**).* Starting with **82** (400 mg, 0.761 mmol), general procedure G was performed followed by general procedure F. The resulting mixture was concentrated to half the original volume, diluted with MeOH (∼15 mL), and neutralized with 1 N aqueous $NaHSO_4$ (pH ∼ 7). This was concentrated, diluted with 1:1 MeOH/DCM (∼20 mL), filtered, and concentrated again to provide the title compound (125 mg, 60% for 2 steps) which was used without further purification. MS (ESI+) m/z 272.5 (M + H).

*Step 3: (±)-N-((1-((5,7-Dimethyl-1H-indol-4-yl)methyl)piperidin-2-yl)methyl)methanesulfonamide (**30**).* To a solution of **83** (110 mg, 0.405 mmol) in DCM (8 mL) at 0 °C was added DIPEA (0.425 mL, 2.43 mmol) followed by MsCl (0.095 mL, 1.22 mmol). The resulting mixture was stirred for 10 min and then diluted with saturated aqueous $NaHCO_3$, warmed to rt, and stirred for 30 more minutes. The mixture was further diluted with DCM and saturated aqueous $NaHCO_3$ and extracted 2× with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The resulting residue was partially purified by FCC (5−15% 2 M NH_3 in MeOH/DCM) and then further purified by RP-HPLC-B to provide the title compound (7 mg, 5%). ^{1}H NMR (400 MHz, CD_3CN) δ ppm 9.16 (br s, 1 H), 7.22 (t, J = 2.8 Hz, 1 H), 6.79 (s, 1 H), 6.62 (dd, J = 3.0, 2.0 Hz, 1 H), 4.77 (br s, 1 H), 4.19 (d, J = 12.9 Hz, 1 H), 3.70 (d, J = 12.9 Hz, 1 H), 3.31−3.18 (m, 1 H), 3.16−3.03 (m, 1 H), 2.86−2.70 (m, 1 H), 2.58−2.51 (m, 1 H), 2.48 (s, 3 H), 2.41 (s, 6 H), 2.38−2.30 (m, 1 H), 1.84−1.25 (m, 6 H). HRMS calcd for $C_{18}H_{28}N_3O_2S$ $(M + H)^+$ 350.1897, found 350.1897.

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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(±)-5,7-Dimethyl-4-((2-(2-(methylsulfonyl)ethyl)piperidin-1-yl)methyl)-1*H*-indole (31). *Step 1: (±)-5,7-Dimethyl-4-((2-(2-(methylsulfonyl)ethyl)piperidin-1-yl)methyl)-1-tosyl-1H-indole (**84**).* To a solution of the TFA salt of (±)-2-(2-(methylsulfonyl)-ethyl)piperidine (**129**; see Supporting Information) (200 mg, 0.65 mmol) in DCM (10 mL) was added 1 N aqueous NaOH (2 mL), and this was stirred vigorously for 10 min. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. This material was subjected to general procedure K with **46** (150 mg, 0.431 mmol) followed by FCC purification (40−57% EtOAc/heptane) to provide the title compound (230 mg, 88%). MS (ESI+) *m/z* 503.3 (M + H).

*Step 2: (±)-5,7-Dimethyl-4-((2-(2-(methylsulfonyl)ethyl)-piperidin-1-yl)methyl)-1H-indole (**31**).* General procedure F was performed on **84** (230 mg, 0.458 mmol). The reaction was diluted with DCM and water and extracted with DCM. Organic layers were combined, dried with Na_2SO_4, filtered, and concentrated, and purified by FCC (10−80% EtOAc/DCM) to provide the title compound (105 mg, 66%). ^{1}H NMR (400 MHz, methylene chloride-d_2) δ 8.10 (br s, 1 H), 7.20 (t, J = 2.8 Hz, 1 H), 6.81 (s, 1 H), 6.72−6.61 (m, 1 H), 4.13 (d, J = 12.6 Hz, 1 H), 3.65 (d, J = 12.6 Hz, 1 H), 3.10−2.87 (m, 2 H), 2.75−2.70 (m, 1 H), 2.68 (s, 3 H), 2.57−2.48 (m, 1 H), 2.44 (s, 3 H), 2.43 (s, 3 H), 2.23 (ddd, J = 11.9, 8.0, 3.3 Hz, 1 H), 2.18−1.97 (m, 2 H), 1.82−1.60 (m, 2 H), 1.57−1.54 (m 1 H), 1.47−1.26 (m, 3 H). HRMS calcd for $C_{19}H_{29}N_2O_2S$ $(M + H)^+$ 349.1944, found 349.1949.

(±)-1-((1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)methyl)urea (32). To a solution of **83** (125 mg, 0.46 mmol) in THF (4 mL) was added isocyanatotrimethylsilane (0.26 mL, 1.67 mmol) in three portions. After 1 h the reaction was diluted with MeOH (5 mL), concentrated, and purified by RP-HPLC-B to provide the title compound (4.5 mg, 3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.81 (br s, 1 H), 7.21 (t, J = 2.7 Hz, 1 H), 6.68 (s, 1 H), 6.52 (dd, J = 3.0, 2.0 Hz, 1 H), 5.83 (br s, 1 H), 5.44 (s, 2 H), 4.15 (d, J = 12.1 Hz, 1 H), 3.45−3.35 (m, 2 H), 3.30−3.18 (m, 2 H), 2.39 (s, 3 H), 2.36 (s, 3 H), 2.32−2.24 (m, 1 H), 2.04−1.84 (m, 1 H), 1.73−1.52 (m, 2 H), 1.47−0.98 (m, 4 H). HRMS calcd for $C_{18}H_{27}N_4O$ $(M + H)^+$ 315.2179, found 315.2174.

(±)-5,7-Dimethyl-4-((2-(4-(methylsulfonyl)phenyl)-piperidin-1-yl)methyl)-1*H*-indole (33). *Step 1: (±)-5,7-Dimethyl-4-((2-(4-(methylsulfonyl)phenyl)piperidin-1-yl)methyl)-1-tosyl-1H-indole (**85**).* General procedure K with (±)-2-(4-(methylsulfonyl)-phenyl)piperidine (**135**; see Supporting Information) (83 mg, 0.347 mmol) and **46** (101 mg, 0.289 mmol) followed by FCC purification (0−80% EtOAc/heptane) provided the title compound (98 mg, 62%). MS (ESI+) *m/z* 551.5 (M + H).

*Step 2: (±)-5,7-Dimethyl-4-((2-(4-(methylsulfonyl)phenyl)-piperidin-1-yl)methyl)-1H-indole (**33**).* General procedure F was performed on **85** (98 mg, 0.178 mmol). The reaction was diluted with water, acidified with 2 N HCl until pH < 7, basified with sodium bicarbonate, and extracted 2× with EtOAc. Organic layers were combined, dried with Na_2SO_4, filtered, concentrated, and purified by FCC (0−100% EtOAc/heptane) to provide the title compound (12 mg, 16%). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.12 (br s, 1H), 7.91 (d, J = 8.34 Hz, 2H), 7.78 (d, J = 8.08 Hz, 2H), 7.22 (dd, J = 2.80, 3.00 Hz, 1H), 6.78 (s, 1H), 6.70 (dd, J = 2.27, 3.03 Hz, 1H), 3.70 (d, J = 12.38 Hz, 1H), 3.27 (d, J = 12.38 Hz, 1H), 3.24−3.20 (m, 1H), 3.06 (s, 3H), 2.82−2.74 (m, 1H), 2.44 (s, 3H), 2.35 (s, 3H), 2.05−1.96 (m, 1H), 1.85−1.75 (m, 2H), 1.75−1.64 (m, 1H), 1.53−1.38 (m, 3H). HRMS calcd for $C_{23}H_{29}N_2O_2S$ $(M + H)^+$ 397.1950, found 397.1936.

(±)-4-(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)benzamide ((±)-34) and (±)-4-(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid ((±)-35). *Step 1: (±)-4-(1-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)methyl)piperidin-2-yl)-benzonitrile (**86**).* General procedure K with 4-(piperidin-2-yl)-benzonitrile hydrochloride (CAS no. 1203685-85-1, 112 mg, 0.600 mmol) and **46** (174 mg, 0.50 mmol) followed by FCC purification (0−100% EtOAc/heptane) provided the title compound (176 mg, 71%). MS (ESI+) *m/z* 498.5 (M + H).

*Step 2: (±)-4-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)piperidin-2-yl)benzamide ((±)-**34**) and (±)-4-(1-((5,7-Dimethyl-1H-indol-4-yl)-methyl)piperidin-2-yl)benzoic Acid ((±)-**35**).* A mixture of **86** (100 mg, 0.201 mmol) and KOH (100 mg, 1.78 mmol) in EtOH (2 mL) was stirred and heated at 100 °C via microwave irradiation for 1 h. The mixture was acidified with AcOH until pH ∼ 6, then purified directly by RP-HPLC-A to provide the first eluting product (±)-**34** (26 mg, 23%) and the second eluting product (±)-**35** (27 mg, 26%). (±)-**34**: ^{1}H NMR (TFA salt, 400 MHz, D_2O) δ 8.02−7.92 (m, 2H), 7.76 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 3.1 Hz, 1H), 6.86 (s, 1H), 6.24 (s, 1H), 4.56−4.47 (m, 1H), 4.32−4.17 (m, 2H), 3.44 (d, J = 12.4 Hz, 1H), 3.29 (t, J = 12.6 Hz, 1H), 2.41 (s, 3H), 2.20−2.03 (m, 5H), 2.00−1.80 (m, 2H), 1.80−1.58 (m, 2H). HRMS calcd for $C_{23}H_{28}N_3O$ $(M + H)^+$ 362.2232, found 362.2221. (±)-**35**: ^{1}H NMR (TFA salt, 400 MHz, methanol-d_4) δ 10.82 (s, 1H), 8.24 (d, J = 8.2 Hz, 2H), 7.76 (d, J = 8.1 Hz, 2H), 7.32 (dt, J = 3.0, 1.3 Hz, 1H), 6.83 (s, 1H), 6.36 (d, J = 3.1 Hz, 1H), 4.58 (dd, J = 10.4, 5.2 Hz, 1H), 4.38−4.18 (m, 2H), 3.53 (d, J = 12.6 Hz, 1H), 3.44−3.34 (m, 1H), 2.45 (s, 3H), 2.24−2.07 (m, 5H), 2.01−1.72 (m, 4H). HRMS calcd for $C_{23}H_{27}N_2O_2$ $(M + H)^+$ 363.2073, found 363.2070.

(−)-4-(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid ((−)-35). *Step 1: (±)-Methyl 4-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)piperidin-2-yl)benzoate ((±)-**87**).* Starting with **86** (550 mg, 1.10 mmol), general procedure L followed by general procedure M was performed followed by FCC purification (24% EtOAc/heptane) to provide the title compound (300 mg, 72% for 2 steps). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ 9.10 (s, 1H), 8.04−7.95 (m, 2H), 7.65 (d, J = 8.0 Hz, 2H), 7.17 (t, J = 2.9 Hz, 1H), 6.71 (s, 1H), 6.58 (dd, J = 3.2, 2.0 Hz, 1H), 3.86 (s, 3H), 3.61 (d, J = 12.1 Hz, 1H), 3.24−3.12 (m, 2H), 2.66 (ddd, J = 10.6, 3.4, 1.8 Hz, 1H), 2.39 (s, 3H), 2.27 (s, 3H), 1.82−1.61 (m, 4H), 1.57−1.39 (m, 3H). MS (ESI+) *m/z* 377.5 (M + H).

*Step 2: Methyl 4-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-piperidin-2-yl)benzoate (**87a**) and Methyl 4-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)piperidin-2-yl)benzoate (**87b**).* Resolution of the enantiomers of (±)-**87** (500 mg, 1.33 mmol) was achieved by chiral SFC using a CHIRALCEL OJ-H column with 30% (0.2% DEA in MeOH) in CO_2 to provide the first eluting enantiomer **87a** (t_r = 2.6 min, 230 mg, 46%) and the second eluting enantiomer **87b** (t_r = 4.1 min, 210 mg, 42%).

*Step 3: (−)-4-(1-((5,7-Dimethyl-1H-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid ((−)-**35**).* A mixture of **87b** (150 mg, 0.398 mmol) and KOH (50 mg, 0.89 mmol) in THF (1 mL) was stirred at rt for 1 h. The mixture was then concentrated and purified by RP-HPLC-B to provide the title compound (120 mg, 83%). $[\alpha]_{25}^{D}$ +5.8° (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 7.95 (d, J = 8.59 Hz, 2H), 7.58 (br d, J = 7.80 Hz, 2H), 7.20 (d, J = 3.00 Hz, 1H), 6.71 (s, 1H), 6.08 (br s, 1H), 4.39−4.32 (m, 1H), 4.13 (d, J = 13.60 Hz, 1H), 4.06 (d, J = 13.60 Hz, 1H), 3.27 (br d, J = 12.40 Hz, 1H), 3.17−3.07 (m, 1H), 2.25 (s, 3H), 2.03−1.92 (m, 5H), 1.82−1.74 (m, 1H), 1.74−1.65 (m, 1H), 1.61−1.44 (m, 2H). HRMS calcd for $C_{23}H_{27}N_2O_2$ $(M + H)^+$ 363.2073, found 363.2067.

(+)-4-(1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid ((+)-35). Starting with **87a** (150 mg, 0.398 mmol), following the same procedure used for ((−)-**35**) provided the title compound (130 mg, 88%). $[\alpha]_{25}^{D}$ −26.5° (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 7.95 (d, J = 8.59 Hz, 2H), 7.58 (br d, J = 7.80 Hz, 2H), 7.20 (d, J = 3.00 Hz, 1H), 6.71 (s, 1H), 6.08 (br s, 1H), 4.39−4.32 (m, 1H), 4.13 (d, J = 13.60 Hz, 1H), 4.06 (d, J = 13.60 Hz, 1H), 3.27 (br d, J = 12.40 Hz, 1H), 3.17−3.07 (m, 1H), 2.25 (s, 3H), 2.03−1.92 (m, 5H), 1.82−1.74 (m, 1H), 1.74−1.65 (m, 1H), 1.61−1.44 (m, 2H). HRMS calcd for $C_{23}H_{27}N_2O_2$ $(M + H)^+$ 363.2073, found 363.2068.

4-((2*S*,4*S*)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoic Acid (36). *Step 1: (±)-4-(trans-1-((5,7-Dimethyl-1-tosyl-1H-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzonitrile (**88**).* General procedure J with (±)-4-(*trans*-4-methoxypiperidin-2-yl)benzonitrile (**140**; see Supporting Information) (170 mg, 0.786 mmol) and **46** (420 mg, 1.21 mmol) was followed. The crude material was cooled to rt, diluted with DCM, filtered, and purified by passage through a cation exchange column (Biotage Isolute SCX-2 column) eluting initially with 20% MeOH/DCM followed by 7 N NH_3 in MeOH to provide the title compound (320 mg, 77%). MS (ESI+) *m/z* 528.5 (M + H).

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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*Step 2: (±)-Methyl 4-(trans-1-((5,7-Dimethyl-1H-indol-4-yl)-methyl)-4-methoxypiperidin-2-yl)benzoate ((±)-**89**).* Starting with **88** (320 mg, 0.606 mmol), general procedure L was followed except that isoamylamine (0.5 mL, 4.3 mmol) was also added to the reaction mixture. General procedure M was then performed, followed by FCC purification (33% EtOAc/heptane) to provide the title compound (35 mg, 14% for 2 steps). ^{1}H NMR (400 MHz, CD_3CN) δ 9.09 (br s, 1H), 7.99 (d, J = 8.34 Hz, 2H), 7.65 (br d, J = 8.10 Hz, 2H), 7.17 (app t, J = 2.78 Hz, 1H), 6.71 (s, 1H), 6.57 (dd, J = 2.02, 3.03 Hz, 1H), 3.85 (s, 3H), 3.60 (d, J = 12.10 Hz, 1H), 3.54−3.45 (m, 2H), 3.29−3.21 (m, 4H), 2.40−2.34 (m, 4H), 2.33−2.23 (m, 4H), 1.91−1.86 (m, 1H), 1.85−1.79 (m, 1H), 1.78−1.70 (m, 1H), 1.56−1.45 (m, 1H). HRMS calcd for $C_{25}H_{31}N_2O_3$ $(M + H)^+$ 407.2335, found 407.2326.

*Step 3: Methyl 4-((2R,4R)-1-((5,7-Dimethyl-1H-indol-4-yl)-methyl)-4-methoxypiperidin-2-yl)benzoate (**89a**) and Methyl 4-((2S,4S)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**89b**).* Resolution of the enantiomers of (±)-**89** (30 mg, 0.074 mmol) was achieved by chiral SFC using a CHIRALCEL OJ-H column with 30% (10 mM NH_4OH in MeOH) in CO_2 to provide the first eluting enantiomer, methyl 4-((2*R*,4*R*)-1-((5,7-dimethyl-1*H*-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**89a**) (t_r = 2.4 min, 12 mg, 40%) and the second eluting enantiomer, methyl 4-((2*S*,4*S*)-1-((5,7-dimethyl-1*H*-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**89b**) (t_r = 3.4 min, 14 mg, 47%). The absolute stereochemistry was determined via chemical correlation to compound **41**.

*Step 4: 4-((2S,4S)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoic Acid (**36**).* General procedure N was performed on **89b** (14 mg, 0.034 mmol) at 70 °C for 1 h, followed by RP-HPLC-B purification to provide the title compound (4 mg, 29%). ^{1}H NMR (400 MHz, D_2O) δ 7.81 (d, J = 8.08 Hz, 2H), 7.47 (d, J = 8.10 Hz, 2H), 7.11 (d, J = 3.03 Hz, 1H), 6.60 (s, 1H), 6.06 (br s, 1H), 3.92 (br s, 1H), 3.70 (d, J = 12.63 Hz, 1H), 3.59 (br s, 1H), 3.53−3.28 (m, 1H), 3.22 (s, 3H), 2.74 (br s, 2H), 2.19 (s, 3H), 2.10−1.94 (m, 2H), 1.88 (s, 3H), 1.84−1.70 (m, 1H), 1.69−1.40 (m, 1H). HRMS calcd for $C_{24}H_{29}N_2O_3$ $(M + H)^+$ 393.2178, found 393.2173.

4-((2*S*,4*S*)-1-((5-Cyclopropyl-7-methyl-1*H*-indol-4-yl)-methyl)-4-methoxypiperidin-2-yl)benzoic Acid (37). *Step 1: (±)-tert-Butyl 5-Cyclopropyl-4-(trans-(4-methoxy-2-(4-(methoxycarbonyl)phenyl)piperidin-1-yl)methyl)-7-methyl-1H-indole-1-carboxylate (**90**).* General procedure J with (±)-methyl 4-(*trans*-4-methoxypiperidin-2-yl)benzoate (**143**; see Supporting Information) (287 mg, 1.15 mmol) and **52** (350 mg, 1.09 mmol) followed by FCC purification (23% EtOAc/heptane) provided the title compound (410 mg, 70%). MS (ESI+) m/z 533.6 (M + H).

*Step 2: (±)-Methyl 4-(trans-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate ((±)-**91**).* General procedure H was performed on **90** (410 mg, 0.770 mmol). The reaction was cooled to rt, acidified with half saturated aqueous citric acid, extracted 3×x with DCM/TFE (9:1), dried with Na_2SO_4, filtered, and concentrated. General procedure M was then performed, followed by FCC purification (21% EtOAc/heptane) to provide the title compound (300 mg, 90% for 2 steps). MS (ESI+) m/z 433.4 (M + H).

*Step 3: Methyl 4-((2S,4S)-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**91a**) and Methyl 4-((2R,4R)-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**91b**).* Resolution of the enantiomers of (±)-**91** (300 mg, 0.69 mmol) was achieved by chiral SFC using a CHIRALCEL OJ-H column with 30% (5 mM NH_4OH in MeOH) in CO_2 to provide the first eluting enantiomer, methyl 4-((2*S*,4*S*)-1-((5-cyclopropyl-7-methyl-1*H*-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**91a**) (t_r = 2.0 min, 100 mg, 28%), and the second eluting enantiomer, methyl 4-((2*R*,4*R*)-1-((5-cyclopropyl-7-methyl-1*H*-indol-4-yl)methyl)-4-methoxypiperidin-2-yl)benzoate (**91b**) (t_r = 4.3 min, 100 mg, 28%). The absolute stereochemistry was determined via chemical correlation to compound **41**.

*Step 4: 4-((2S,4S)-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)-methyl)-4-methoxypiperidin-2-yl)benzoic Acid (**37**).* General procedure N was performed on **91a** (100 mg, 0.23 mmol) at 70 °C for 8 h, followed by RP-HPLC-B purification to provide the title compound (59 mg, 61%). ^{1}H NMR (400 MHz, D_2O) δ 7.81 (br d, J = 8.30 Hz, 2H), 7.51 (br d, J = 7.80 Hz, 2H), 7.20 (d, J = 3.28 Hz, 1H), 6.52 (s, 1H), 6.16 (br s, 1H), 4.46−4.15 (m, 2H), 3.97 (br s, 1H), 3.66 (br s, 1H), 3.24 (s, 3H), 3.20−2.96 (m, 2H), 2.23 (s, 3H), 2.14 (br s, 2H), 1.90 (br d, J = 15.40 Hz, 1H), 1.70 (br s, 1H), 1.45 (br s, 1H), 0.66 (br s, 1H), 0.55 (br s, 1H), 0.14 (br s, 1H), −0.11 (br s, 1H). HRMS calcd for $C_{26}H_{31}N_2O_3$ $(M + H)^+$ 419.2335, found 419.2335.

4-((2*S*,4*S*)-4-Methoxy-1-((5-methoxy-7-methyl-1*H*-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid (38). *Step 1: (±)-tert-Butyl 5-Methoxy-4-(trans-(4-methoxy-2-(4-(methoxycarbonyl)-phenyl)piperidin-1-yl)methyl)-7-methyl-1H-indole-1-carboxylate ((±)-**92**).* General procedure O with **57** (120 mg, 0.415 mmol) and (±)-methyl 4-(*trans*-4-methoxypiperidin-2-yl)benzoate (**143**; see Supporting Information) (100 mg, 0.401 mmol), followed by FCC purification on aminopropyl-functionalized silica gel (6% EtOAc/heptane), provided the title compound (110 mg, 52%). MS (ESI+) m/z 523.4 (M + H).

*Step 2: tert-Butyl 5-Methoxy-4-((2R,4R)-(4-methoxy-2-(4-(methoxycarbonyl)phenyl)piperidin-1-yl)methyl)-7-methyl-1H-indole-1-carboxylate (**92a**) and tert-Butyl 5-Methoxy-4-((2S,4S)-(4-methoxy-2-(4-(methoxycarbonyl)phenyl)piperidin-1-yl)methyl)-7-methyl-1H-indole-1-carboxylate (**92b**).* Resolution of the enantiomers of (±)-**92** (110 mg, 0.21 mmol) was achieved by chiral SFC using a CHIRALPAK AD-H column with 15% (5 mM NH_4OH in MeOH) in CO_2 to provide the first eluting enantiomer, *tert*-butyl 5-methoxy-4-((2*R*,4*R*)-(4-methoxy-2-(4-(methoxycarbonyl)phenyl)-piperidin-1-yl)methyl)-7-methyl-1*H*-indole-1-carboxylate (**92a**) (t_r = 2.8 min, 35 mg, 29%), and the second eluting enantiomer, *tert*-butyl 5-methoxy-4-((2*S*,4*S*)-(4-methoxy-2-(4-(methoxycarbonyl)phenyl)-piperidin-1-yl)methyl)-7-methyl-1*H*-indole-1-carboxylate (**92b**) (t_r = 5.5 min, 30 mg, 25%). The absolute stereochemistry was determined via chemical correlation to compound **41**.

*Step 3: 4-((2S,4S)-4-Methoxy-1-((5-methoxy-7-methyl-1H-indol-4-yl)methyl)piperidin-2-yl)benzoic Acid (**38**).* General procedure N was performed on **92b** (30 mg, 0.057 mmol) at 70 °C for 4 h, followed by RP-HPLC-B purification to provide the title compound (19 mg, 79%). ^{1}H NMR (400 MHz, D_2O) δ 7.99 (d, J = 8.10 Hz, 2H), 7.63 (br d, J = 8.10 Hz, 2H), 7.34 (d, J = 3.03 Hz, 1H), 6.80 (s, 1H), 6.30 (d, J = 3.03 Hz, 1H), 4.02−3.79 (m, 2H), 3.79−3.73 (m, 1H), 3.69 (s, 3H), 3.49−3.29 (m, 4H), 2.89 (br d, J = 10.90 Hz, 1H), 2.83−2.63 (m, 1H), 2.45 (s, 3H), 2.20−2.07 (m, 1H), 2.06−1.88 (m, 2H), 1.87−1.61 (m, 1H). HRMS calcd for $C_{24}H_{29}N_2O_4$ $(M + H)^+$ 409.2127, found 409.2119.

4-((2*S*,4*S*)-1-((5,7-Dimethyl-1*H*-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoic Acid (39). *Step 1: (±)-tert-Butyl 4-(trans-(4-Ethoxy-2-(4-(methoxycarbonyl)phenyl)piperidin-1-yl)methyl)-5,7-dimethyl-1H-indole-1-carboxylate (**53**).* General procedure K with (±)-**50** (300 mg, 1.14 mmol) and **51** (368 mg, 1.25 mmol) followed by FCC purification (0−80% EtOAc/heptane) provided the title compound (530 mg, 89%). MS (ESI+) m/z 521.6 (M + H).

*Step 2: (±)-Methyl 4-(trans-1-((5,7-Dimethyl-1H-indol-4-yl)-methyl)-4-ethoxypiperidin-2-yl)benzoate ((±)-**55**).* General procedure H was performed on **53** (530 mg, 1.02 mmol). The reaction was cooled to rt, diluted with water, and immediately neutralized with 1 N HCl until pH < 7, then basified with sodium bicarbonate, extracted 3× with EtOAc, dried with Na_2SO_4, filtered, and concentrated. General procedure M was then performed, followed by FCC purification (0−100% EtOAc/heptane) to provide the title compound (350 mg, 82% for 2 steps). MS (ESI+) m/z 421.8 (M + H).

*Step 3: Methyl 4-((2S,4S)-1-((5,7-Dimethyl-1H-indol-4-yl)-methyl)-4-ethoxypiperidin-2-yl)benzoate (**55a**) and Methyl 4-((2R,4R)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoate (**55b**).* Resolution of the enantiomers of (±)-**55** (350 mg, 0.83 mmol) was achieved by chiral SFC using a CHIRALPAK AD-H column with 40% (5 mM NH_4OH in ^{i}PrOH) in CO_2 to provide the first eluting enantiomer, methyl 4-((2*S*,4*S*)-1-((5,7-dimethyl-1*H*-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoate (**55a**) (t_r = 1.7 min, 164 mg, 47%), and the second eluting enantiomer, methyl 4-((2*R*,4*R*)-1-((5,7-dimethyl-1*H*-indol-4-yl)-methyl)-4-ethoxypiperidin-2-yl)benzoate (**55b**) (t_r = 4.4 min, 161

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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mg, 46%). The absolute stereochemistry was determined via chemical correlation to compound **41**.

*Step 4: 4-((2S,4S)-1-((5,7-Dimethyl-1H-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoic Acid (**39**).* General procedure N was performed on **55a** (86 mg, 0.20 mmol) at rt for 16 h, followed by RP-HPLC-A purification to provide the title compound as the TFA salt (101 mg, 90%). ^{1}H NMR (TFA salt, 600 MHz, D_2O) δ 8.04 (d, J = 7.79 Hz, 2H), 7.73 (br d, J = 7.40 Hz, 2H), 7.38 (d, J = 2.84 Hz, 1H), 6.89 (s, 1H), 6.29 (br s, 1H), 4.64 (br s, 1H), 4.28 (br s, 1H), 4.13 (br s, 1H), 3.97 (br s, 1H), 3.65 (q, J = 6.94 Hz, 2H), 3.40 (br s, 1H), 3.22 (br s, 1H), 2.45 (s, 3H), 2.34 (br s, 2H), 2.15 (br s, 3H), 2.11–2.03 (m, 1H), 1.90 (br s, 1H), 1.29 (t, J = 7.02 Hz, 3H). HRMS calcd for $C_{25}H_{31}N_2O_3$ $(M + H)^+$ 407.2335, found 407.2332.

4-((2*S*,4*S*)-1-((5-Cyclopropyl-7-methyl-1*H*-indol-4-yl)-methyl)-4-ethoxypiperidin-2-yl)benzoic Acid (40). *Step 1: (±)-tert-Butyl 5-Cyclopropyl-4-(trans-(4-ethoxy-2-(4-(methoxycarbonyl)phenyl)piperidin-1-yl)methyl)-7-methyl-1H-indole-1-carboxylate (**54**).* General procedure K with (±)-**50** (256 mg, 0.972 mmol) and **52** (342 mg, 1.07 mmol) followed by FCC purification (0–80% EtOAc/heptane) provided the title compound (450 mg, 85%). MS (ESI+) m/z 547.3 (M + H).

*Step 2: (±)-Methyl 4-(trans-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoate ((±)-**56**).* General procedure H was performed on **54** (450 mg, 0.82 mmol). The reaction was cooled to rt, diluted with water, and immediately neutralized with 1 N HCl until pH < 7, then basified with sodium bicarbonate, extracted 3× with EtOAc, dried with Na_2SO_4, filtered, and concentrated. This was purified by FCC (0–100% EtOAc/heptane) to provide the title compound (230 mg, 63%). MS (ESI+) m/z 447.5 (M + H).

*Step 3: Methyl 4-((2S,4S)-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoate (**56a**) and Methyl 4-((2R,4R)-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoate (**56b**).* Resolution of the enantiomers of (±)-**56** (265 mg, 0.59 mmol) was achieved by chiral SFC using a CHIRALPAK AD-H column with 40% (5 mM NH_4OH in ^{i}PrOH) in CO_2 to provide the first eluting enantiomer, methyl 4-((2*S*,4*S*)-1-((5-cyclopropyl-7-methyl-1*H*-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)-benzoate (**56a**) (t_r = 1.3 min, 84 mg, 32%), and the second eluting enantiomer, methyl 4-((2*R*,4*R*)-1-((5-cyclopropyl-7-methyl-1*H*-indol-4-yl)methyl)-4-ethoxypiperidin-2-yl)benzoate (**56b**) (t_r = 2.9 min, 69 mg, 26%). The absolute stereochemistry was determined via chemical correlation to compound **41**.

*Step 4: 4-((2S,4S)-1-((5-Cyclopropyl-7-methyl-1H-indol-4-yl)-methyl)-4-ethoxypiperidin-2-yl)benzoic Acid (**40**).* General procedure N was performed on **56a** (84 mg, 0.19 mmol) at rt for 16 h, followed by RP-HPLC-A purification to provide the title compound as the TFA salt (72 mg, 68%). ^{1}H NMR (600 MHz, D_2O) δ 8.03 (d, J = 8.25 Hz, 2H), 7.71 (br d, J = 7.80 Hz, 2H), 7.41 (d, J = 2.93 Hz, 1H), 6.71 (s, 1H), 6.32 (br s, 1H), 4.73–4.63 (m, 1H), 4.52 (d, J = 12.30 Hz, 1H), 4.30 (d, J = 12.30 Hz, 1H), 3.98 (br s, 1H), 3.65 (q, J = 7.00 Hz, 2H), 3.56–3.42 (m, 1H), 3.34 (br d, J = 11.00 Hz, 1H), 2.43 (s, 3H), 2.40–2.26 (m, 2H), 2.10 (d, J = 15.31 Hz, 1H), 1.92 (br s, 1H), 1.61 (br s, 1H), 1.29 (t, J = 7.00 Hz, 3H), 0.87 (br s, 1H), 0.76 (br s, 1H), 0.34 (br s, 1H), 0.08 (br s, 1H). HRMS calcd for $C_{27}H_{33}N_2O_3$ $(M + H)^+$ 433.2491, found 433.2482.

4-((2*S*,4*S*)-(4-Ethoxy-1-((5-methoxy-7-methyl-1*H*-indol-4-yl)methyl)piperidin-2-yl))benzoic Acid (41, LNP023). *Step 1: tert-Butyl 4-(((2S,4S)-4-Ethoxy-2-(4-(methoxycarbonyl)phenyl)-piperidin-1-yl)methyl)-5-methoxy-7-methyl-1H-indole-1-carboxylate (**58**).* To a solution of *tert*-butyl 4-formyl-5-methoxy-7-methyl-1*H*-indole-1-carboxylate (**57**) (1.5 g, 5.18 mmol) and methyl 4-((2*S*,4*S*)-4-ethoxypiperidin-2-yl)benzoate ((2*S*,4*S*)-**50**) (1.185 g, 4.50 mmol) in DCE (20 mL) was added $NaBH(OAc)_3$ (3 g, 14.1 mmol), and this was stirred at rt for 21.5h. Additional *tert*-butyl 4-formyl-5-methoxy-7-methyl-1*H*-indole-1-carboxylate (**57**) (500 mg, 1.90 mmol) was added, and this was stirred for 20 h. The reaction was diluted with EtOAc, washed successively with 5% aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated to provide the title compound (2.415 g, quant) which was used without further purification. MS (ESI+) m/z 537.4 (M + H). The absolute stereochemistry was ultimately determined via cocrystallization of **41** with the catalytic domain of FB.

*Step 2: 4-((2S,4S)-(4-Ethoxy-1-((5-methoxy-7-methyl-1H-indol-4-yl)methyl)piperidin-2-yl))benzoic Acid (**41**, LNP023).* To a solution of *tert*-butyl 4-(((2*S*,4*S*)-4-ethoxy-2-(4-(methoxycarbonyl)phenyl)-piperidin-1-yl)methyl)-5-methoxy-7-methyl-1*H*-indole-1-carboxylate (**58**) (2.415 g, 4.50 mmol) in THF (10 mL) and MeOH (20 mL) was added 1 M LiOH in H_2O (15 mL, 15 mmol), and this was stirred at 70 °C for 8 h. The reaction was cooled to rt, diluted with H_2O, half saturated aqueous $KHSO_4$ and citric acid, saturated with sodium chloride, then extracted with 9:1 DCM/TFE, dried with Na_2SO_4, filtered, and concentrated. RP-HPLC-B purification provided the title compound (730 mg, 38% for 2 steps). ^{1}H NMR (400 MHz, D_2O) δ 7.96 (d, J = 8.0 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 3.2 Hz, 1H), 6.66 (s, 1H), 6.20 (s, 1H), 4.62–4.47 (m, 1H), 4.06 (d, J = 13.2 Hz, 1H), 3.97–3.76 (m, 2H), 3.66–3.48 (m, 5H), 3.43–3.29 (m, 1H), 3.26–3.15 (m, 1H), 2.35 (s, 3H), 2.31–2.11 (m, 2H), 2.00 (d, J = 15.4 Hz, 1H), 1.93–1.74 (m, 1H), 1.25–1.07 (m, 3H). HRMS calcd for $C_{25}H_{31}N_2O_4$ $(M + H)^+$ 423.2284, found 423.2263.

4-((2*S*,4*S*)-(4-Ethoxy-1-((5-methoxy-7-methyl-1*H*-indol-4-yl)methyl)piperidin-2-yl))benzoic Acid Hydrochloride (41·HCl). To a solution of **41** (620 mg, 1.47 mmol) in H_2O (10 mL) and acetonitrile (3 mL) was added 5 M aqueous HCl (0.5 mL, 2.5 mmol). The mixture was then lyophilized, and the resulting solid was suspended in ^{i}PrOH and heated to 70 °C. The mixture turned into a solution after 1.5 h and was then cooled to rt with stirring. After about 5 h, the mixture turned into a suspension and the solid was collected by filtration and dried under high vacuum at 50 °C to provide the title compound as the hydrochloride salt (450 mg, 65%). ^{1}H NMR (400 MHz, methanol-d_4) δ 10.73 (s, 1H), 8.23 (d, J = 8.2 Hz, 2H), 7.74 (d, J = 8.3 Hz, 2H), 7.36–7.31 (m, 1H), 6.77 (s, 1H), 6.42–6.31 (m, 1H), 4.40–4.19 (m, 2H), 3.87–3.80 (m, 1H), 3.76 (s, 3H), 3.68–3.50 (m, 4H), 3.45–3.38 (m, 1H), 2.51 (s, 3H), 2.30–2.18 (m, 2H), 2.13–1.89 (m, 2H), 1.31 (t, J = 7.0 Hz, 3H). MS (ESI+) m/z 423.3 (M + H).

2. Protein Crystallography. Cocrystallization screening experiments were conducted with all compounds by using the catalytic domain (Asp470-Leu764) of human FB at a concentration of 25 mg/mL in 20 mM Tris, pH 7.5, containing 100 mM NaCl. Crystals were grown at 20 °C using a sitting drop vapor diffusion format. All crystals were cryocooled in liquid nitrogen without the addition of cryoprotectant, and data were collected at the PXII beamline of the Swiss Light Source (SLS) at the Paul Scherrer Institute (PSI). Crystal structures were solved by molecular replacement using the published structure of the catalytic domain (Protein Data Bank code 1DLE). Atomic coordinates and structure factors for the crystal structures of FB with compounds **1**, **8**, (*S*)-**19**, **29**, **35**, and **41** are deposited in the Protein Data Bank with accession codes 6QSW.pdb, 6T8U.pdb, 6T8W.pdb, 6QSX.pdb, 6T8V.pdb, and 6RAV.pdb, respectively. Authors will release the atomic coordinates and experimental data upon article publication.

3. Biological and in Vivo Experiments. The procedures for the CVF-Bb ELISA assessments, FB-comp FRET based assay, 50% human serum MAC deposition, and 50% mouse serum C3b deposition assays, as well as SPR determinations have been reported previously and can be found in ref 13. The experimental protocol for the mouse LPS challenge model has previously been described in ref 9. In all cases protocols, handling, and care of animals were in accordance with the policy of the NIBR Cambridge Animal Care and Use Committee.

3.1. Plasma Pharmacokinetic Studies in Mice. The pharmacokinetics of compounds **35**–**38**, **40**, and **41** were determined in C57BL/6 mice. The compounds were dosed intravenously (iv, via injection into jugular vein, 1 mg/kg, n = 2 animals/compound) and orally (po, via oral gavage, 10 mg/kg, n = 3 animals/compound). The iv solution formulations were prepared at 1 mg/mL as described in the parentheses for each compound: **35**, **36**, and **41** (10% propylene glycol, 25% solution of 20% solutol, and phosphate buffered saline), **37** (1 equiv of 1 N sodium hydroxide solution, 10% propylene glycol, 25% solution of 20% solutol, and phosphate buffered saline, and 1 N

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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hydrochloric acid for pH adjustment), **38** (1 equiv of 1 N hydrochrolic acid, 30% polyethylene glycol 300, 50% solution of 20% chremophor EL, and phosphate buffered saline), **40** (2 equiv of 1 N sodium hydroxide solution, 10% propylene glycol, 25% solution of 20% Solutol, and phosphate buffered saline and 1 N hydrochloric acid for pH adjustment). The po formulations were suspensions in 0.5% methylcellulose and 0.5% Tween 80. Approximately 50 μL of whole blood was collected from the tails at 5 min (iv dose only), 15 min (po dose only), 0.5 h, 1 h, 2 h, 4 h, and 7 h postdose and was transferred to EDTA tubes. Blood was centrifuged at 3000 rpm, and the resultant plasma was transferred to a capped PCR 96-well plate and frozen at −20 °C until subsequent analysis by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC−MS/MS). Chromatographic separation was carried out on ACE C18 column (30 mm × 2.1 mm, 50 μm) (MAC-MOD Analytical, Chadds Ford, PA), using a gradient elution method with water and acetonitrile, both containing 0.1% formic acid. Mass spectrometric measurements in positive electrospray ionization were directed at quantifying the respective $[M + H]^+$ precursor ions to the relevant fragment ions on Sciex (Sciex, Framingham, MA) or Thermo (Thermo, Waltham, MA) mass spectrometers. The relevant pharmacokinetic parameters were estimated using noncompartmental methods using WinNonlin (Enterprise, version 5.2) purchased from Pharsight Corporation (St. Louis, MO) or Watson LIMS (Thermo, Waltham, MA).

3.2. Plasma Pharmacokinetic Studies in Rats. The pharmacokinetics of compound **41** was determined in Wistar Han rats. The compound was dosed intravenously (iv, via injection into the jugular vein catheter; 1 mg/kg, $n = 2$ animals) and orally (po, via oral gavage; 30 mg/kg, $n = 2$ animals). The same solution formulation for both iv and po routes of administration was employed and was prepared in phosphate buffered saline containing 2 equiv of 1 N hydrochloric acid, 30% polyethylene glycol 300, and 50% of a 20% Cremophor EL solution, and phosphate buffered saline. Approximately 200 μL of whole blood was collected from the tails at 5 min (iv dose only), 15 min, 0.5 h, 1 h, 2 h, 4 h, 7 h, and 24 h postdose and was transferred to EDTA tubes. Blood was centrifuged at 3000 rpm, and the resultant plasma was transferred to a capped PCR 96-well plate and frozen at −20 °C until subsequent analysis by HPLC−MS/MS. Chromatographic separation was carried out on ACE C18 column (30 mm × 2.1 mm, 50 μm) (MAC-MOD Analytical, Chadds Ford, PA), using a gradient elution method with water and acetonitrile, both containing 0.1% formic acid. Mass spectrometric measurements in positive electrospray ionization were directed at quantifying the mass transition with $[M + H]^+$ as the precursor ion, namely, 423 → 117, and 423 → 115 on either Sciex (Sciex, Framingham, MA) or Thermo (Thermo, Waltham, MA) mass spectrometers. The relevant pharmacokinetic parameters were estimated using noncompartmental methods using WinNonlin (Enterprise, version 5.2) purchased from Pharsight Corporation (St. Louis, MO) or Watson LIMS (Thermo, Waltham, MA).

3.3. Ocular Pharmacokinetic Studies in Rats. Three-month-old brown Norway rats were administered **41** via oral gavage as a suspension in 0.5% hydroxypropyl methylcellulose and 0.1% Tween 80. Ocular tissues from both eyes and plasma were collected from two rats per time point at 0.25, 0.5, 1, 3, 6, and 24 h after administration. The ocular tissues collected were the retina and the posterior eye cup (RPE/choroid and posterior sclera). The tissues were diluted with phosphate buffered saline containing 10% acetonitrile and homogenized, centrifuged prior to analyses. The concentrations of the test article were measured in plasma and supernatants of ocular homogenates by HPLC−MS/MS in four individual retinas, four individual posterior eye cups, and two individual plasma samples at each time point. Chromatographic separation was carried out on ACE C18 column (30 mm × 2.1 mm, 50 μm) (MAC-MOD Analytical, Chadds Ford, PA), using a gradient elution method with water and acetonitrile, both containing 0.1% formic acid. Mass spectrometric measurements in positive electrospray ionization were directed at quantifying the mass transition with $[M + H]^+$ as the precursor ion, namely, 423 → 117 and 423 → 115 on either Sciex mass spectrometer (Sciex, Framingham, MA). The relevant pharmacokinetic parameters were estimated using noncompartmental methods using WinNonlin (Enterprise, version 5.2) purchased from Pharsight Corporation (St. Louis, MO) or Watson LIMS (Thermo, Waltham, MA).

3.4. Plasma Pharmacokinetic Studies in Dogs. The pharmacokinetics of compound **41** as an HCl salt was determined in male beagle dogs. The compound was dosed intravenously (0.1 mg/kg, $n = 3$ animals) and orally (10 mg/kg, $n = 3$ animals/compound) by gavage. The iv formulation was a solution consisting of 10% *N*-methyl-2-pyrrolidone and 90% polyethylene glycol 200, whereas the po formulation was a solution consisting of 0.5% methylcellulose and 0.5% Tween 80 in water. Blood was collected at 5 min (iv dose only), 15 min, 0.5 h, 1 h, 2 h, 4 h, 7 h, and 24 h postdose. The blood samples were diluted with acetonitrile and were analyzed by HPLC−MS/MS under electrospray ionization in positive mode. Chromatographic separation was carried out on Phenomenex KINETEX C18 column (50 mm × 2.1 mm, 2.6 μm) (Phenomenex, Basel, Switzerland), using a gradient elution method with water and methanol, both containing 1% formic acid. Mass spectrometric measurements in positive electrospray ionization were directed at quantifying the mass transition with $[M + H]^+$ as the precursor ion, namely, 423→ 117 and 423→ 115 on Sciex 5500 mass spectrometer (Sciex, Darmstadt, Germany). Similarly, the relevant pharmacokinetic parameters were estimated using noncompartmental methods using WinNonlin (Enterprise, version 5.2) purchased from Pharsight Corporation (St. Louis, MO) or Watson LIMS (Thermo, Waltham, MA). Other relevant calculations were performed in Microsoft Excel.

3.5. Ex Vivo Dog MAC Deposition Assay. Compound **41** as an HCl salt was administered as a solution consisting of 0.5% methylcellulose and 0.5% Tween 80 in water to beagle dogs ($n = 4$). Serum samples from all animals were collected predose and at 0.1, 0.25, 0.5, 1, 2, 4, 7, 10, 12, 24, 30, and 36 h postdose and stored at −80 °C. A 384-well transfer plate was prepared with dog serum samples (15 μL per well, $n = 3$) and prediluted (1/1, v/v) in ice-cold 0.15 mM $CaCl_2$, 141 mM NaCl, 4.5 mM $MgCl_2$, 0.1% gelatin, 4.2 mM HEPES buffer, pH 7.4, containing purified human complement C9 (3 nM) and 20 mM EGTA (alternative pathway not blocked but classical and lectin pathways blocked) or purified human complement C9 (3 nM) and 20 mM EDTA (baseline control, all complement activation pathways blocked). A volume of 25 μL of serum plus buffer mixture was subsequently transferred to the zymosan-coated reaction plate. The plate was centrifuged (1 min, 1000 rpm), mixed (2 min), sealed with a plastic adhesive film, and placed at 37 °C for 15 min. The reaction was terminated by aspirating the samples. Wells were saturated with blocking buffer (100 μL/well, 10 min, rt) and then washed 3 times with TBS-T (100 μL/well). Detection of MAC complex deposited on the plate was achieved by ELISA using as primary antibody a mouse anti-human C9 neo-epitope monoclonal antibody (0.25 μg/mL in TBS-T, 25 μL/well, 60 min at RT) followed after washing 3 times with TBS -T (100 μL) by a horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (1/1000 dilution in TBS-T, 25 μL/well, 45 min at rt). After washing 3 times with TBS-T (100 μL), QuantaBlu fluorogenic peroxidase substrate (25 μL/well) was added to the plate and the HRP was allowed to develop for 10 min at rt. Plates were read at 325 nm excitation/420 nm emission in a TECAN Safire2 fluorimeter. The raw data obtained were exported into an Excel file. Excel was used to calculate averages of the triplicate wells for each sample. The baseline (EDTA-treated serum, the negative control) and the maximum (EGTA-pretreatment serum (time 0), the positive control) were used to generate a % inhibition value for each of the serum samples tested (formula 1):

$$\%\ \text{inhibition} = \frac{(\text{maximum average}) - (\text{baseline average})}{(\text{test well average}) - (\text{baseline average})} \times 100 \quad (1)$$

https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.9b01870.

Molecular formula strings with CVF-Bb or FB-comp IC_{50} data and human and mouse 50% serum IC_{50} data (CSV)

Detailed chemistry experimentals for intermediates and details related to in vitro selectivity profiling; crystallographic structure determination information on FB with compounds **8**, (*S*)-**19**, and **35**. Crystallographic information for compounds **1**, **29**, and **41** has been reported previously.[13] (PDF)

Accession Codes

Atomic coordinates and structure factors for the crystal structures of FB with compounds **1**, **8**, (*S*)-**19**, **29**, **35**, and **41** are deposited in the Protein Data Bank with accession codes 6QSW.pdb, 6T8U.pdb, 6T8W.pdb, 6QSX.pdb, 6T8V.pdb, and 6RAV.pdb, respectively. Authors will release the atomic coordinates and experimental data upon article publication.

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https://dx.doi.org/10.1021/acs.jmedchem.9b01870
J. Med. Chem. XXXX, XXX, XXX−XXX

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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.

■ ACKNOWLEDGMENTS

We thank Jay Larrow, Scott Plummer, and Carina Sanchez for their contributions to large scale preparations of **41**; Ann Brown for bioanalytical support; Andreas Boettcher for biochemical and cellular assays in support; Rene Assenberg, Claude Logel, Daniele Dietschin, and Ingrid Bechtold for support in protein production; and Christopher Towler, Lipa Shah, and Geeti Gangal for preparation of compound formulations for in vivo studies. All work described was funded by Novartis Institutes for BioMedical Research Inc.

■ ABBREVIATIONS USED

aHUS, atypical hemolytic uremic syndrome; AMD, age-related macular degeneration; AP, alternative complement pathway; BRB, blood−retinal barrier; C3G, C3 glomerulopathy; CVF, cobra venom factor; CVF-Bb, cobra venom factor Bb complex; DCE, 1,2-dichloroethane; DIPEA, *N*,*N*-diisopropylethylamine; DMC-Cl, chloro-1,3-dimethylimidazolinium chloride; FB, complement factor B; FB-comp, TR-FRET based competition FB binding assay; FCC, flash column chromatography; FD, complement factor D; FH, complement factor H; FI, complement factor I; MAC, membrane attack complex; PNH, paroxysmal nocturnal hemoglobinuria; RPE, retinal pigment epithelial; SEM, [2-(trimethylsilyl)ethoxy]methyl acetal; SFC, supercritical fluid chromatography; SPD, serine protease domain; TBPDS, *tert*-butyldiphenylsilyl

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(42) A large set of compounds from a different subseries than what is disclosed in this manuscript were assessed in both the CVF-Bb and FB-comp assays, and the resulting IC_{50} values showed good concordance enabling a direct comparison or potency between the two assay formats.
(43) The cocrystal of **29** with FB illustrated in Figure 5 provides a different perspective of the structure previously reported in ref 13.
(44) The cocrystal structure indicated that the stereochemistry of the phenylpiperidine was of the *S* configuration in line with the preferred enantiomers (*S*-)-**19** and **29**. Thus it is likely that the absolute stereochemistry of (−)-**35** is also *S*; however since the racemic **35** was used for the crystallization experiment, this cannot be concluded unambiguously.
(45) The absolute stereochemistry was determined via chemical correlation once the cocrystal structure was ascertained for compound **41**.
(46) Figure 7 provides a different perspective of the cocrystal structure previously reported in ref 13.
(47) A list of proteins evaluated can be found in the Supporting Information.
(48) The plasma data represented in Figure 8 have previously been disclosed in ref 13, while ocular complement inhibition is a new disclosure.
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https://dx.doi.org/10.1021/acs.jmedchem.9b01870
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binding kinetics. Binding parameters B_{max} and K_d were calculated by a Langmuir type of multiple-site linear regression analysis. B_{max} refers to the maximal binding capacity of a particular binding site of melanin and K_d to the respective dissociation constant. These parameters are indexed 1 and 2 for low and high affinity sites, respectively. **41** affords B_{max1} and B_{max2} values of 32 and 4.9 nmol/mg, respectively, and K_{d1} and K_{d2} values of 26.0 and 2.0 μM.

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