Journal of Medicinal Chemistry

pubs.acs.org/jmc

Article

Design and Synthesis of Novel Spiro Derivatives as Potent and Reversible Monoacylglycerol Lipase (MAGL) Inhibitors: Bioisosteric Transformation from 3-Oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl Moiety

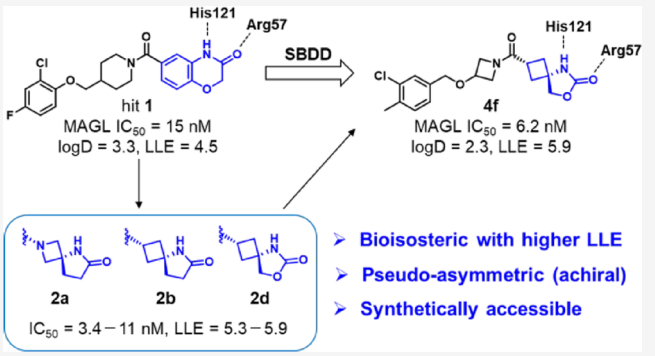
Shuhei Ikeda,* Hideyuki Sugiyama, Hidekazu Tokuhara, Masataka Murakami, Minoru Nakamura, Yuya Oguro, Jumpei Aida, Nao Morishita, Satoshi Sogabe, Douglas R. Dougan, Sean C. Gay, Ling Qin, Naoto Arimura, Yasuko Takahashi, Masako Sasaki, Yusuke Kamada, Kazunobu Aoyama, Kouya Kimoto, and Makoto Kamata

Cite This: https://doi.org/10.1021/acs.jmedchem.1c00432

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The therapeutic potential of monoacylglycerol lipase (MAGL) inhibitors in central nervous system-related diseases has attracted attention worldwide. However, the availability of reversible-type inhibitor is still limited to clarify the pharmacological effect. Herein, we report the discovery of novel spiro chemical series as potent and reversible MAGL inhibitors with a different binding mode to MAGL using Arg57 and His121. Starting from hit compound **1** and its co-crystal structure with MAGL, structure-based drug discovery (SBDD) approach enabled us to generate various spiro scaffolds like **2a** (azetidine-lactam), **2b** (cyclobutane-lactam), and **2d** (cyclobutane-carbamate) as novel bioisosteres of 3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl moiety in **1** with higher lipophilic ligand efficiency (LLE). Optimization of the left hand side afforded **4f** as a promising reversible MAGL inhibitor, which showed potent in vitro MAGL inhibitory activity (IC_{50} 6.2 nM), good oral absorption, blood−brain barrier penetration, and significant pharmacodynamic changes (2-arachidonoylglycerol increase and arachidonic acid decrease) at 0.3−10 mg/kg, po. in mice.



INTRODUCTION

The endocannabinoid signaling system, naturally modulated by two endocannabinoids (ECs), 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (anandamide), via the activation of cannabinoid receptors (CB_1 and CB_2), regulates numerous physiological processes such as pain perception, appetite, learning and memory, and reward behaviors.[1] CB_1 receptor is highly expressed in the central nervous system (CNS) and less so in peripheral tissues, whereas CB_2 receptor is predominantly expressed in immune cells, including microglia in the brain. Among ECs, 2-AG, a major contributor in the brain,[2−4] is degraded to arachidonic acid (AA) and glycerol primarily by monoacylglycerol lipase (MAGL),[5,6] a serine hydrolase abundant in brain tissue (Figure 1A).[7−9] Therefore, MAGL inhibition causes an elevation in 2-AG levels as well as a depletion in AA levels in the brain, which enhances endocannabinoid signaling and causes antineuroinflammatory effects.[10] Based on these observations, intensive investigations on MAGL inhibition have been conducted[11−14] to determine its therapeutic potential for various related diseases such as Parkinson's and Alzheimer's diseases, multiple sclerosis, depression, and epilepsy.[15,16]

Two types of MAGL inhibitors have been reported to date (Figure 1B). Among them, irreversible ones such as compound **a** (JZL184) and compound **b** (ABX-1431) have been extensively used to confirm the beneficial biological effects of MAGL inhibition on CNS disease pathologies and ABX-1431 has been evaluated in human clinical trials.[17−20] In the investigations, it was reported that the chronic blockade of MAGL using a genetic method or chemical one with JZL184 caused undesired responses such as pharmacological tolerance and CB_1 receptor desensitization.[20] Thus, reversible

Received: March 10, 2021

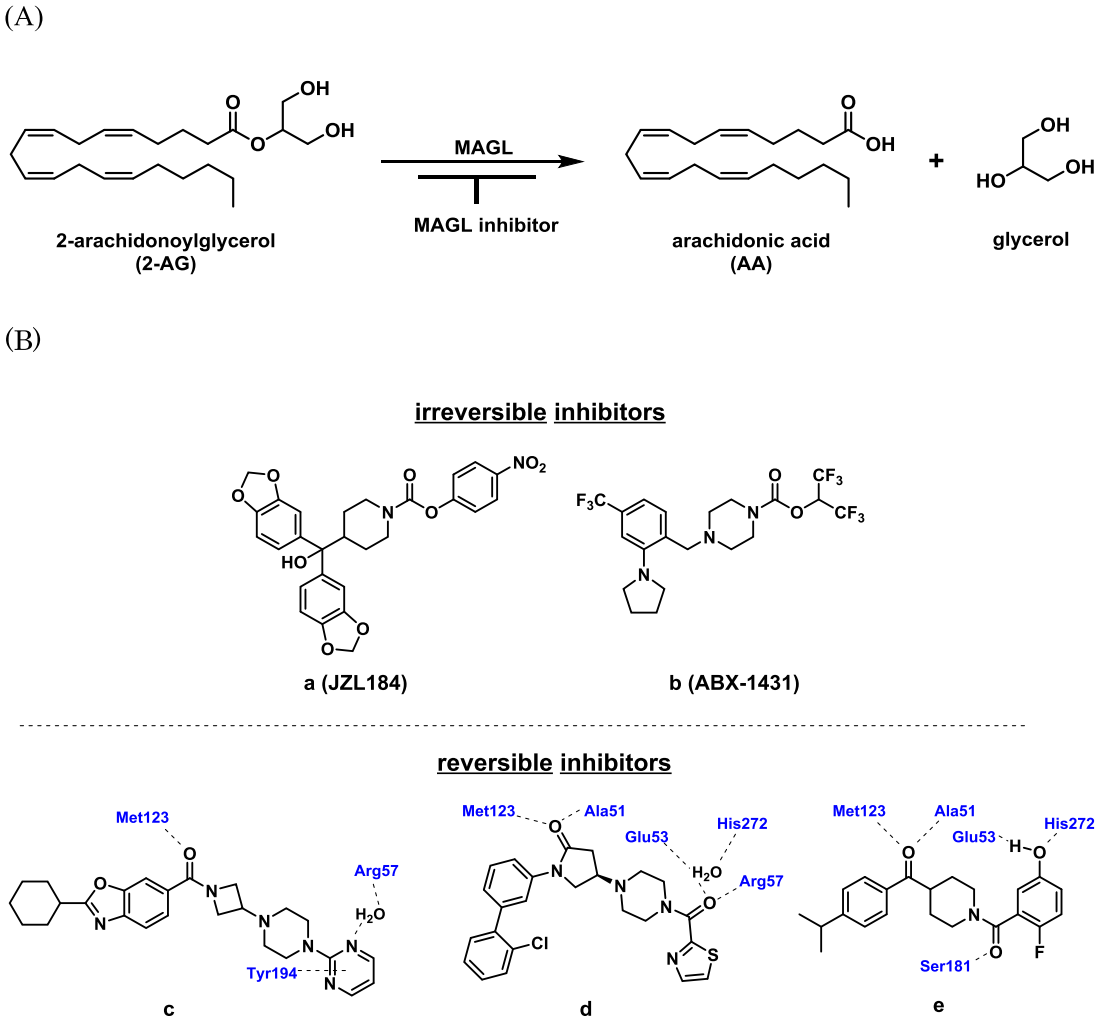


Figure 1. (A) Enzymatic reaction by MAGL. (B) Representative MAGL inhibitors, irreversible type: compounds **a** (JZL184)[20] and **b** (ABX-1431);[17] reversible type: compounds **c** (PDB ID 3PE6),[29] **d** (PDB ID 5ZUN),[21] and **e** (predicted complex).[22a] Major amino acids in MAGL catalytic site are illustrated in blue, which form polar interactions with compounds **c**–**e**.

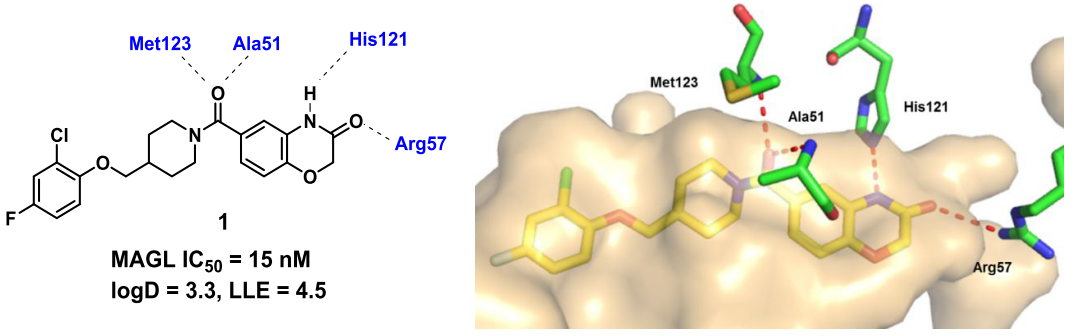


Figure 2. HTS hit **1** and co-crystal structure in the active site of MAGL (yellow, PDB ID 7L4T).

inhibitors have been expected to circumvent these potential disadvantages and some chemical series represented by compounds **c**,[29] **d**,[21] and **e**[22a] were reported to show potent in vitro MAGL inhibitory activities as well as efficacy in models of neuropathic and inflammatory pain.[21−25] Regarding compounds **c** and **d**, their co-crystal structures with human MAGL well illustrate the key binding features for the potent activities. That is, the central amide carbonyl group recruits Met123 and Ala51, the right half of the molecules forms hydrogen bonds with Arg57, His272, and Glu53 in a direct or water-mediated manner, and the left half occupies the binding pocket by their rather lipophilic structures. In spite of these great inventions, as far as we know, none of the reversible inhibitors entered the clinical trial. Therefore, further expansion of the chemical space in reversible inhibitors is considered necessary to clarify the positive pharmacological effects of MAGL inhibition.

Based on these backgrounds, we aimed at generating novel, potent, and reversible MAGL inhibitors, which show different binding modes to the target utilizing structure-based drug

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

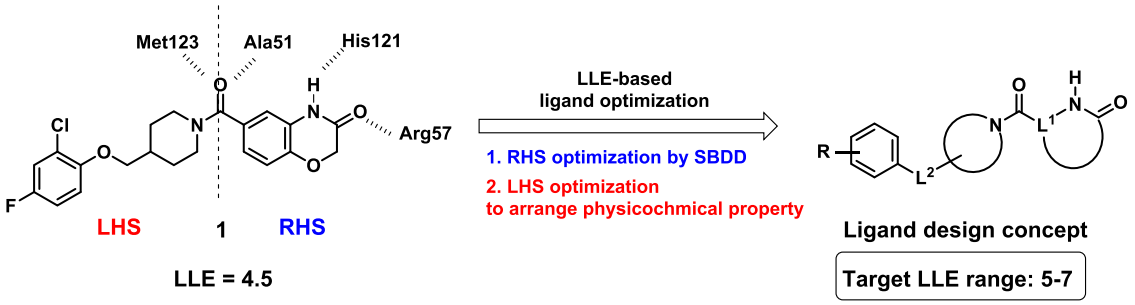
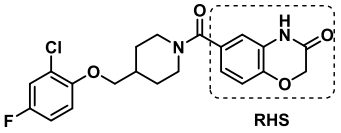
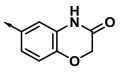
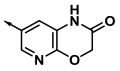
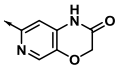
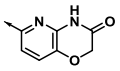
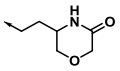
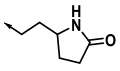
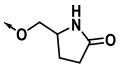
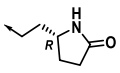
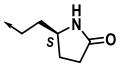


Figure 3. LLE/SBDD-based ligand optimization strategy.

Table 1. First Stage of RHS Optimization



Cpd	RHS	MAGL[a] IC_{50} (nM)[b]	log D[c]	LLE[d]	MDR BA/AB[e]
1		15 (9.9-22)	3.3	4.5	0.7
1a		26 (22-31)	2.6	5.0	1.8
1b		27 (19-38)	3.0	4.6	0.7
1c		32 (7-38)	2.6	4.9	2.5
1d		1100 (860-1400)	2.6	3.4	1.1
1e		63 (49-82)	2.7	4.6	1.7
1f		190 (150-240)	3.0	3.8	0.6
1g		590 (410-860)	2.7	3.6	1.3
1h		38 (32-45)	2.7	4.8	1.5
JZL184		1100 (900-1300)			

[a]Inhibitory activity against human MAGL enzyme. [b]IC_{50} values and 95% confidence intervals (in parentheses) were calculated from duplicate measurements using a four-parameter logistic model. [c]Measured at pH 7.4.[27] [d]LLE = pIC_{50} − log *D*.[26] [e]MDR1 efflux ratios in P-gp-overexpressing cells at 1 μM of substrate.

discovery (SBDD) approach. To obtain a high-quality MAGL inhibitor considering its physicochemical properties, we also used lipophilic ligand efficiency (LLE)[26] as an indicator throughout our research because this strategy could provide a

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

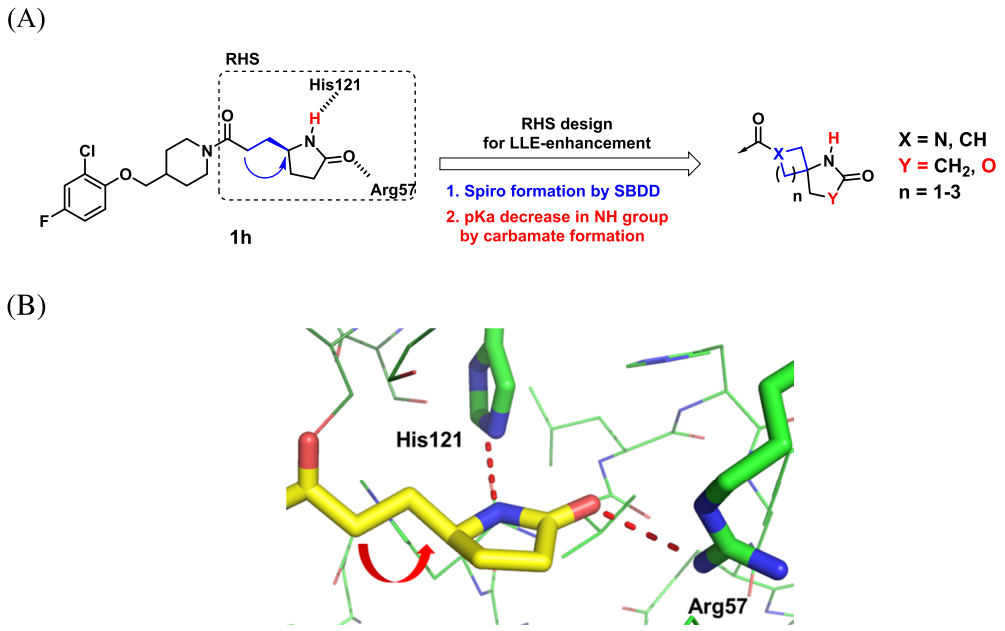


Figure 4. (A) Spiro formation from compound **1h** and pK_a tuning strategy. (B) RHS in the co-crystal structure of compound **1h** with MAGL (yellow, PDB ID 7L4U).

selective ligand to MAGL without too much addition of lipophilicity, which often causes nonspecific bindings to off-targets. Herein, we report an LLE/SBDD-based lead generation/optimization approach from hit compound **1**, which leads to the discovery of (2*s*,4*s*)-2-((3-((3-chloro-4-methylbenzyl)oxy)azetidin-1-yl)carbonyl)-7-oxa-5-azaspiro-[3.4]octan-6-one (**4f**) as a novel, potent, and reversible MAGL inhibitor.

RESULTS AND DISCUSSION

Using high-throughput screening (HTS), we identified compound **1**, with potent MAGL inhibitory activity (IC_{50} = 15 nM) and moderate LLE (LLE[26] = $pIC_{50} - \log D$[27] = 4.5; Figure 2). The crystal structures of human MAGL have been disclosed as apo form and in complex with reversible and irreversible inhibitors.[21,28,29] Our co-crystallization experiments successfully yielded high-quality crystals and the density maps contained clear features of **1** in the active site of MAGL. Examination of the co-crystal structure revealed a novel binding mode to MAGL and critical aspects of interaction as follows. The central amide carbonyl oxygen atom points into the oxyanion hole[29] and forms hydrogen bonds with the backbone amide NHs from residues Ala51 and Met123 adjacent to the catalytic Ser122, suggesting a noncovalent reversible mode of inhibition. On the right-hand side (RHS) moiety, 3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazin-6-yl, the carbonyl oxygen atom forms a hydrogen bond with the side chain of Arg57 and the NH group forms a hydrogen bond with the side chain of His121. These two direct hydrogen bonds formed between our ligand and MAGL protein are unique considering the other binding modes of MAGL inhibitors reported so far. On the left-hand-side (LHS) moiety, ((2-chloro-4-fluorophenoxy)methyl)-piperidine, it packs a relatively wider region with the lipophilic substituents. These effective binding interactions well explain the strong inhibitory activity of compound **1** (IC_{50} = 15 nM).

The analysis on the co-crystal structure inspired us to think of the ligand optimization strategy as follows (Figure 3). The essential central carbonyl group should be kept in the designed structure to maintain MAGL inhibitory activity. In addition, LLE should be monitored to see if we are moving in the right direction toward the final target range of 5−7, which is considered as an optimal range using oral drug data analysis.[26] As a first step, the RHS optimization was planned by SBDD because the two hydrogen bonds with His121 and Arg57 would be the key contributors to the potency and LLE of compound **1**, considering the strength of the polar interactions.[30] For the RHS, we designed a cyclic unit with amide functionality and a linker (L^1) that can interact with His121 and Arg57. It was assumed that a tactful linker (L^1) design by SBDD would be the key to enhance LLE. In the second stage, arrangement of the physicochemical properties was planned via modification of the LHS moiety while maintaining LLE. As indicated in the co-crystal structure, the wider space around the LHS moiety would allow us to investigate various types of LHS design, including different ring sizes of the amine portion, various types of linkers (L^2), and substituents on the benzene ring.

Optimization of RHS Using SBDD. In the first step of RHS optimization, we tried to enhance LLE by incorporating an N atom into the benzene ring and removing the benzene moiety itself (Table 1). The structural modification of the benzene spacer was expected to be tolerable because no beneficial molecular interaction was observed in the co-crystal structure of **1** with MAGL. The synthesized compounds were narrowed down to a promising lead candidate in light of LLE and MDR1 efflux ratio (MDR BA/AB, human) as a surrogate of blood−brain barrier (BBB) permeability.[31] Compared to that of compound **1** (IC_{50} = 15 nM, LLE = 4.5), pyridine derivatives **1a**−**c** showed a slight loss of MAGL inhibitory activity (IC_{50} = 26−32 nM), but all showed the LLE enhancement (4.6−5.0). Among them, **1a** displayed the highest LLE (5.0) as well as an acceptable MDR1 efflux ratio (1.8) less than 2.[31]

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

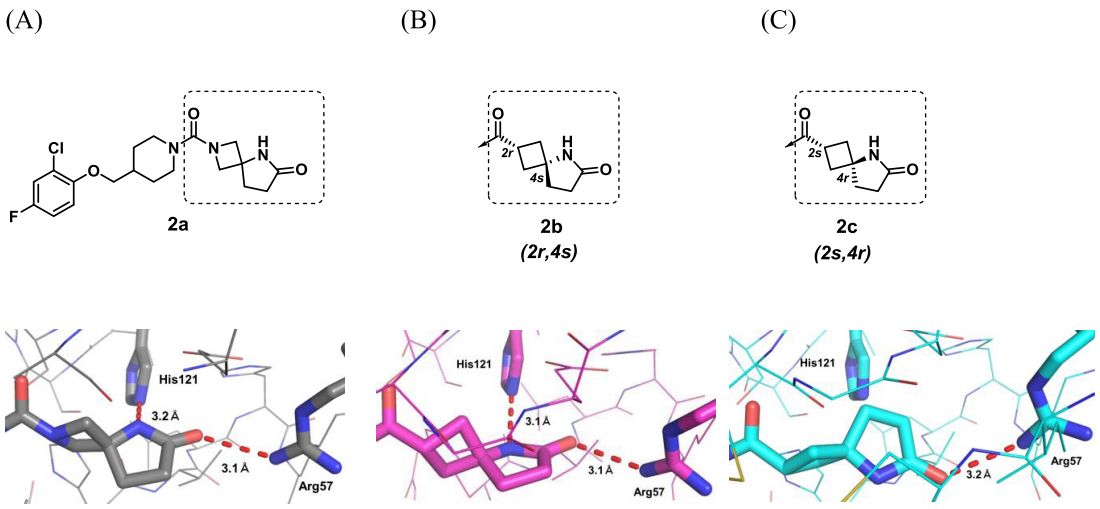
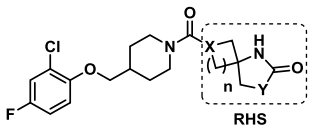
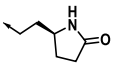
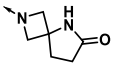
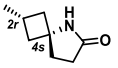
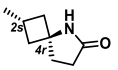
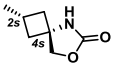
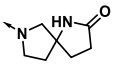
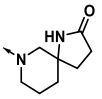


Figure 5. Docking models of the designed spiro compounds using co-crystal structure of **1h** (PDB ID 7L4U) as a template. (A) Spiroazetidine **2a** (gray), (B) (2*r*,4*s*)-spirocyclobutane **2b** (magenta), and (C) (2*s*,4*r*)-spirocyclobutane **2c** (cyan).

Table 2. Second Stage of RHS Optimization



Cpd	RHS	MAGL[a] IC_{50} (nM)[b]	log D[c]	LLE[d]	MDR BA/AB[e]	pKa[f]
1h		38 (32-45)	2.7	4.8	1.5	16.5
2a		11 (7.3-16)	2.7	5.3	4.3	16.3
2b		6.5 (6.1-7.0)	2.8	5.4	1.8	16.1
2c		890 (670-1200)	2.8	3.3	4.4	16.1
2d		3.4 (2.7-4.4)	2.6	5.9	1.6	12.2
2e		73 (49-110)	2.8	4.3	5.3	16.4
2f		>10000	3.1	<2.9	ND[g]	16.5

[a]Inhibitory activity against human MAGL enzyme. [b]IC_{50} values and 95% confidence intervals (in parentheses) were calculated from duplicate measurements using the four-parameter logistic model. [c]Measured at pH 7.4.[27] [d]LLE = pIC_{50} − log *D*.[26] [e]MDR1 efflux ratios in P-gp-overexpressing cells at 1 μM of substrate. [f]pK_a values were calculated by ACD Labs. [g]Not determined.

By removing the RHS benzene ring from **1**, the resultant morpholinone derivative **1d** showed a 10-fold decrease in potency (IC_{50} = 1100 nM), whereas the lactam derivative **1e** also showed a 4-fold decreased potency (IC_{50} = 63 nM) but maintained the LLE (4.6) in comparison with that of **1**. These data suggested that the fixation of the terminal amide portion by the benzene spacer would be critical to form the hydrogen bonds with His121 and Arg57 effectively. Between

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

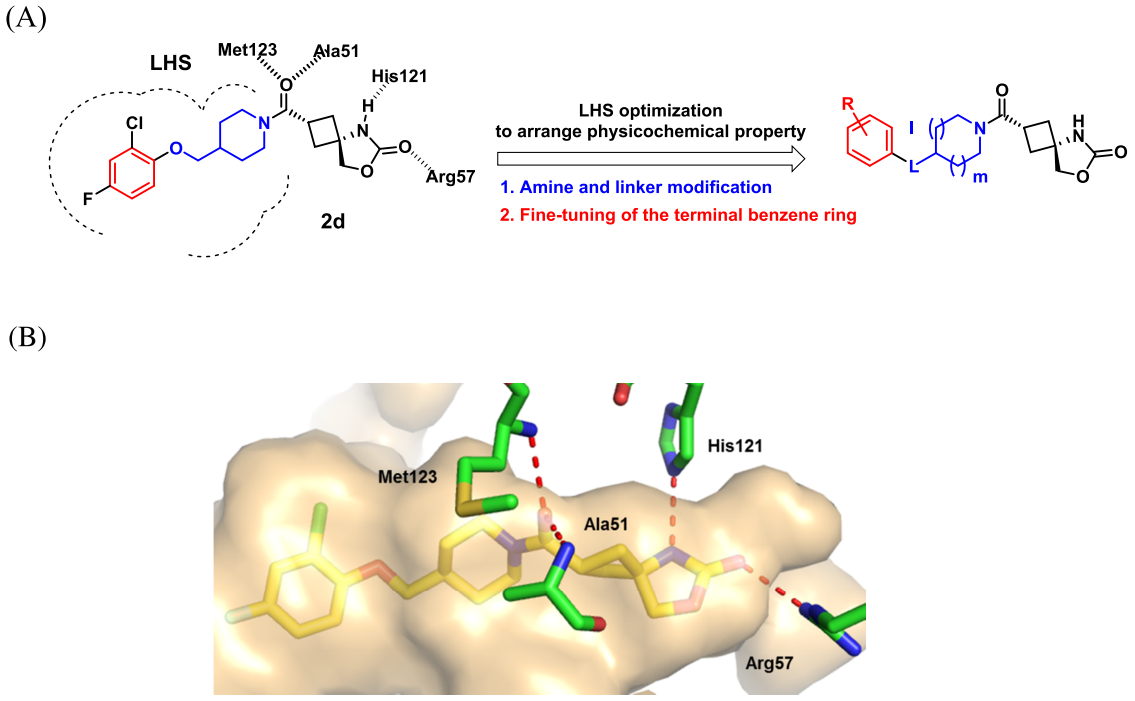


Figure 6. (A) LHS optimization strategy from compound **2d**. (B) Co-crystal structure of **2d** with MAGL (yellow, PDB ID 7L4W).

1d and **1e**, the more rigid five-membered ring conformation of **1e** could afford less discrepancy between the active binding conformation and the most stable ligand conformation. Replacing the ethylene spacer of **1e** with oxymethylene one decreased potency (**1f**, IC_{50} = 190 nM) and LLE (3.8). One reason for this result would be a slight conformational change by changing the spacer which affects the formation of the hydrogen bonds with His121 and Arg57. In addition, the decreased electron density on the O atom in the central carbonyl group might be another reason by the transformation from amide **1e** to carbamate **1f**, where the hydrogen bonds would be formed with Ala51 and Met123. After the chiral separation of **1e**, we obtained compound **1h** as the eutomer (S-form) which showed potent MAGL inhibitory activity (IC_{50} = 38 nM) with a high LLE (4.8) and an acceptable MDR1 efflux ratio (1.5). The co-crystal structure of **1h** with MAGL assured the formation of two hydrogen bonds with His121 and Arg57 by its lactam portion (Figure 4B).

Thus, we identified two types of lead candidates such as **1a** and **1h**. Although **1a** exhibited slightly better LLE (5.0 vs 4.8), we selected **1h** as the lead compound for further optimization because **1h** had a greater potential to boost LLE than **1a** using the SBDD approach (Figure 4A). More specifically, the co-crystal structure inspired us to design a spiro formation at its ethylene spacer to enhance potency and LLE by fixing the active conformation of **1h** (Figure 4B). In addition, the acidity increase (pK_a decrease) in the NH group was expected to strengthen the hydrogen bond with His121 by replacing amide with carbamate.

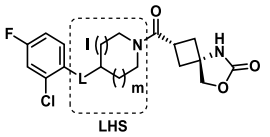
Before taking on the synthesis, we conducted docking studies of the designed spiro compounds (**2a**−**c**) using the co-crystal structure of **1h** (Figure 5). As a result, it was suggested that spiroazetidine **2a** and (2*r*,4*s*)-spirocyclobutane **2b** could efficiently bind to the active site of MAGL by forming two hydrogen bonds with His121 and Arg57 at appropriate distances and angles (Figure 5A,B). However, (2*s*,4*r*)-spirocyclobutane **2c**, stereoisomer of **2b**, was suggested to lose the interaction with His121 in a plausible active conformation (Figure 5C).

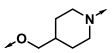
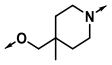
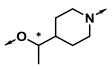
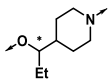
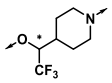
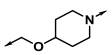
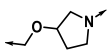
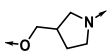
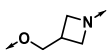
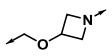
Next, we evaluated the synthesized compounds **2a**−**f** (Table 2). As simulated in the docking studies, compounds **2a** (IC_{50} = 11 nM, LLE = 5.3) and **2b** (IC_{50} = 6.5 nM, LLE = 5.4) showed drastic increases in potency and LLE from **1h**, whereas **2c** (IC_{50} = 890 nM) showed a sharp drop of potency. The stereochemistries of **2b** and **2d** were confirmed by 2D NMR analyses. Between **2a** and **2b**, we selected **2b** because it showed acceptable MDR1 efflux ratio (1.8) and then further modified its lactam portion to the cyclic carbamate of **2d**, following our pK_a tuning strategy. As expected, potency and LLE boosts were observed in **2d** (IC_{50} = 3.4 nM, LLE = 5.9) which was possibly due to a stronger interaction with His121 formed by the more acidic hydrogen. Actually, the pK_a of the carbamate NH group in **2d** is calculated as 12.2 which is lower than 16.1 in lactam **2b**. Finally, we concluded that the four-membered spiro spacer was optimal (*n* = 1, **2a**: IC_{50} = 11 nM) considering the potencies of five-membered (*n* = 2, **2e**: IC_{50} = 73 nM) and six-membered (*n* = 3, **2f**: IC_{50} > 10000 nM) compounds, which indicated that the compact azetidine spacer fixed the active conformation of **1h** in an efficient manner to form the two key interactions with His121 and Arg57.

Optimization of LHS to Arrange the Physicochemical Properties. Through in vitro profiling of compound **2d**, human ether-a-go-go (hERG) liability was found to be addressed (autopatch clamp, 91% inhibition at 10 μM without bovine serum albumin [BSA]). Since the co-crystal structure of **2d** with MAGL also indicated a wider space around the LHS portion like hit compound **1**, we conducted the optimization of amine portion, linker moiety (L), and the substituents on the benzene ring as initially planned (Figure 6A,B). Regarding the terminal aryl ring, it was assumed that

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 3. First Stage of LHS Optimization at the Spacer Portion



Cpd	LHS	MAGL[a] IC_{50} (nM)[b]	log D[c]	LLE[d]	MDR BA/AB[e]	hERG (% inhibition)[f]
2d		3.4 (2.7-4.4)	2.6	5.9	1.6	91.0
3a		2.7 (2.4-3.1)	2.9	5.6	1.9	80.0
3b		2.7 (2.5-3.0)	2.8	5.8	1.2	43.3
3c		2.8 (2.5-3.2)	3.1	5.4	1.7	38.3
3d		4.2 (3.3-5.2)	2.8	5.6	2.7	36.6
3e		11 (9.6-12)	2.5	5.5	1.4	66.4
3f		58 (52-63)	2.1	5.2	2.0	18.6
3g		38 (35-42)	2.2	5.2	2.7	68.1
3h		29 (26-33)	1.8	5.8	2.5	33.7
3i		34 (30-39)	1.9	5.5	1.8	20.1

[a]Inhibitory activity against human MAGL enzyme. [b]IC_{50} values and 95% confidence intervals (in parentheses) were calculated from duplicate measurements using the four-parameter logistic model. [c]Measured at pH 7.4.[27] [d]LLE = pIC_{50} − log *D*.[26] [e]MDR1 efflux ratios in P-gp-overexpressing cells at 1 μM of substrate. [f]Autopatch clamp of hERG inhibition at 10 μM of substrate.

the benzene one should be selected exclusively because our ligand already included two amide moieties as well as one hydrogen bond donor. That is, an extra hetero atom incorporation was not appropriate in this chemical space to obtain a potent MAGL inhibitor with good BBB permeability.[32] During the optimization phase, we primarily aimed at the improvement of hERG liability while keeping high LLE and good MDR1 efflux ratio.

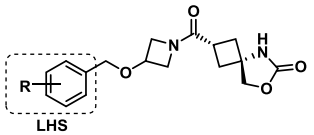
The incorporation of a methyl group at the 4-position in the piperidine ring increased the potency (**3a**, IC_{50} = 2.7 nM), but strong hERG inhibition remained (80.0% at 10 μM; Table 3). Interestingly, the transposition of the methyl group to the adjacent methylene linker led to a significant decrease in hERG liability while maintaining the potency (**3b**, eutomer, IC_{50} = 2.7 nM, hERG = 43.3% at 10 μM). This tendency was also observed in the other branched compounds with different sizes of the substituent (**3c**: ethyl, eutomer; and **3d**: trifluoromethyl, eutomer).

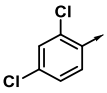
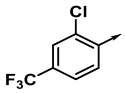
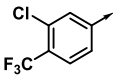
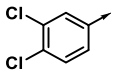
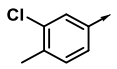
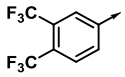
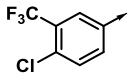
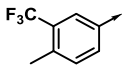
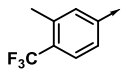
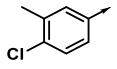
Replacing oxymethylene linker in **2d** with methyleneoxy one caused a slight loss of potency, but the hERG liability showed a sign of improvement (**3e**, IC_{50} = 11 nM, hERG = 66.4% at 10 μM). Eventually, we combined these two linkers (oxymethylene and methyleneoxy) with the different amine rings (pyrrolidine and azetidine) to afford compounds **3f−i**. As a result, a drastic improvement of hERG liability in **3f** and **3i** was observed (**3f**: 18.6% at 10 μM, **3i**: 20.1% at 10 μM), compared to 91.0% at 10 μM in lead compound **2d**. As in the case of **3e**, methyleneoxy linker showed decreased hERG liability in each pair of the molecules, pyrrolidines **3f** (18.6%) and **3g** (68.1%), azetidines **3h** (33.7%) and **3i** (20.1%). Between **3f** and **3i**, compound **3i** was selected for the next stage because of its better LLE (5.5) and MDR1 efflux ratio (1.8).

In the second stage of LHS optimization (Table 4), the effect of the substituents at the 4-position of the benzene ring with 2-Cl substituent was initially investigated (**4a−c**).

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Second Stage of LHS Optimization at the Terminal Benzene Ring



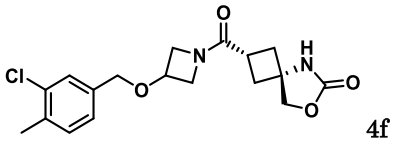
Cpd	LHS	MAGL[a] IC_{50} (nM)[b]	log D[c]	LLE[d]	MDR BA/AB[e]	hERG (% inhibition)[f]
3i		34 (30-39)	1.9	5.5	1.8	20.1
4a		18 (17-19)	2.3	5.5	2.0	16.8
4b		7.0 (6.6-7.3)	2.5	5.6	1.2	46.3
4c		5.4 (4.9-5.9)	2.6	5.6	1.2	43.0
4d		3.2 (3.0-3.5)	2.4	6.1	1.6	42.5
4e		4.4 (4.0-4.7)	2.4	6.0	2.1	56.0
4f		6.2 (5.6-6.9)	2.3	5.9	1.2	18.1
4g		3.8 (3.6-4.0)	2.5	5.9	4.3	46.6
4h		4.1 (3.6-4.8)	2.4	6.0	5.6	38.7
4i		6.3 (5.9-6.7)	2.4	5.8	2.1	27.1
4j		5.4 (5.0-5.9)	2.4	5.9	2.7	39.3
4k		7.1 (6.6-7.7)	2.4	5.8	1.9	20.4

[a]Inhibitory activity against human MAGL enzyme. [b]IC_{50} values and 95% confidence intervals (in parentheses) were calculated from duplicate measurements using the four-parameter logistic model. [c]Measured at pH 7.4.[27] [d]LLE = pIC_{50} − log *D*.[26] [e]MDR1 efflux ratios in P-gp-overexpressing cells at 1 μM of substrate. [f]Autopatch clamp of hERG inhibition at 10 μM of substrate.

Compared to **3i** (IC_{50} = 34 nM), potency enhancement was observed as the lipophilicity of the substituent increased (Me of **4a**: IC_{50} = 18 nM < Cl of **4b**: IC_{50} = 7.0 nM < CF_3 of **4c**: IC_{50} = 5.4 nM), which suggested that the substituent at the 4-position was important for the modulation of MAGL inhibitory activity. Therefore, we also evaluated the potency

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 5. In Vitro Profile Summary of 4f



4f

physicochemical property	selectivity	binding profiles[g] to MAGL[b]
M_w = 364.8	MAGL[b] IC_{50} (nM) = 6.2 (5.6–6.9)	K_D (nM) = 1.5 (±0.33)
log D = 2.3 LLE = 5.9 for MAGL	FAAH[c] IC_{50} (nM) > 10000	k_{on} (M^{-1} s^{-1}) = 2.5 × 10^6 (±2.3×10^5)
	ABHD6[d] IC_{50} (nM) > 10000	k_{off} (s^{-1}) = 0.0036 (±0.0006)
F_{sp^3} = 0.56	hERG[e] inh. at 10 μM = 14.4%	$t_{1/2}$ (min) = 3.3
aqueous solubility[a] = 330 μg/mL	CB_1 inh. 19%[f] at 10 μM CB_2 inh. 5%[f] at 10 μM	

[a]Measured in JP2 (pH 6.8) at 37 °C, JP2: second dissolution fluid in the Japanese Pharmacopoeia.[34] [b]Inhibitory activity against human recombinant MAGL. IC_{50} values and 95% confidence intervals (in parentheses) were calculated from duplicate measurements using the four-parameter logistic model. [c]Inhibitory activity against human recombinant FAAH.[35] [d]Inhibitory activity against human ABHD6. [e]Manual patch clamp of hERG inhibition at 10 μM of substrate. [f]Inhibitory activity against human cannabinoid receptors at 10 μM of substrate. The assay was performed by Eurofins Panlabs Taiwan, Ltd. (https://www.eurofinsdiscoveryservices.com/). [g]K_D, k_{on}, and k_{off} values were derived from surface plasmon resonance (SPR) experiments. The values were expressed as mean ± S.D. (n = 6).

changes with 3,4-disubstituted analogs. The translocation of the Cl atom of **4c** (IC_{50} = 5.4 nM, LLE = 5.6) to the 3-position afforded **4d** with enhanced potency and LLE (IC_{50} = 3.2 nM, LLE = 6.1). Encouraged by this positive result, other 3,4-disubstituted compounds were synthesized in the combination with Me, Cl, and CF_3 substituent. As a result, all of the synthesized compounds **4e–k** showed the higher LLEs (5.8–6.0) in comparison with **3i** (LLE = 5.5). Among them, **4f** (3-Cl, 4-Me) provided the most balanced profiles in terms of potency (IC_{50} = 6.2 nM), LLE (5.9), MDR efflux ratio (1.2), and hERG liability (18.1% at 10 μM). Thus, compound **4f** was selected for further detailed profiling.

In Vitro Profiles of 4f. The low hERG liability (18.1% at 10 μM) of **4f** in the screening autopatch clamp assay was supported by the manual patch clamp assay (14.4% inhibition at 10 μM), which suggested a low risk of cardiovascular QTc prolongation (Table 5). To confirm the MAGL selectivity of **4f**, the inhibitory activities of the most closely related serine hydrolases, such as fatty acid amide hydrolase (FAAH)[11] and α,β-hydrolase domain 6 (ABHD6),[6] were also evaluated. Compound **4f** exhibited no inhibition of these enzymes (IC_{50} > 10000 nM), which indicated that **4f** would enhance endocannabinoid signaling mostly by the increase in the level of 2-AG via selective MAGL inhibition in the brain. In addition, the off-target binding potential to cannabinoid receptors (CB_1 and CB_2) were evaluated at Eurofins Panlabs Taiwan, Ltd. Compound **4f** showed less than 50% inhibition at 10 μM (CB_1: 19% and CB_2: 5%), which suggested that compound **4f** would not interfere the endocannabinoid signaling in a direct manner.

Regarding the physicochemical properties of **4f**, remarkable thermodynamic aqueous solubility (330 μg/mL, pH 6.8) was observed, which would be beneficial in terms of preclinical in vivo evaluation and clinical development. This feature is well explained by its F_{sp^3} rich (F_{sp^3} = 0.56) structure[33] and low lipophilicity (log D = 2.3) which came from our LLE/SBDD-oriented strategy. In particular, the removal of the extra aromatic ring from hit **1** (F_{sp^3} = 0.33, log D = 3.3) and spiro design for lead **2d** (F_{sp^3} = 0.58, log D = 2.6) would be the key steps to reach a chemical structure such as **4f**.

The binding profile of **4f** with MAGL using surface plasmon resonance (SPR) experiments showed a dissociation rate constant (k_{off}) of 0.0036 s^{-1} and dissociation half-life ($t_{1/2}$) of 3.3 min, which indicated that **4f** is a reversible MAGL inhibitor. Furthermore, the reversibility of **4f** from MAGL was tested using the method of the dilution assay previously reported.[21] Recovery of the enzymatic activity after rapid dilution was measured after incubation of MAGL in the absence (control) or presence of **4f**. The activity recovery rate was comparable to that without the inhibitor. On the other hand, the activity recovery was much slower when using an irreversible inhibitor, JZL184. The result also supports that **4f** is a reversible inhibitor. Finally, the co-crystal structure of **4f** was obtained successfully and it displayed a similar binding mode to that of **2d** (Supporting Information, Figure S2).

In Vivo Profiles of 4f. The effect of **4f** on the increase in the level of 2-AG and the decrease in that of AA in the mouse brain was evaluated at 1 h after the oral administration of 0.1–10 mg/kg doses (**4f**: mouse MAGL IC_{50} [nM] = 13). Compound **4f** displayed linear pharmacokinetics in plasma and dose-dependent pharmacokinetics in the brain with good BBB penetration, which would be supported by its excellent solubility and acceptable MDR1 efflux ratio (Table 6).

Table 6. Plasma/Brain Concentration of 4f in Mice at 0.1–10 mg/kg, po.[a]

	concentration (ng/mL or ng/g)	
dose (mg/kg)	plasma	brain
0.1	17	31
0.3	62	99
1	163	169
3	457	322
10	1515	711

[a]Evaluated at 1 h after oral administration (n = 4).

Consistent with the brain concentration, compound **4f** resulted in a significant elevation in the level of 2-AG and reduction in that of AA from 0.3 mg/kg (Figure 7). These in vitro and in vivo results suggested that compound **4f** could be a suitable agent for the validation of diverse therapeutic applications exerted by the reversible MAGL inhibition.

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX–XXX

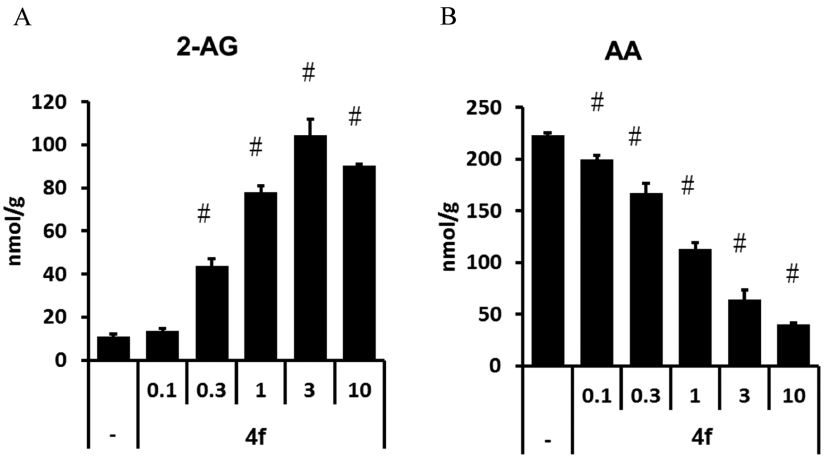
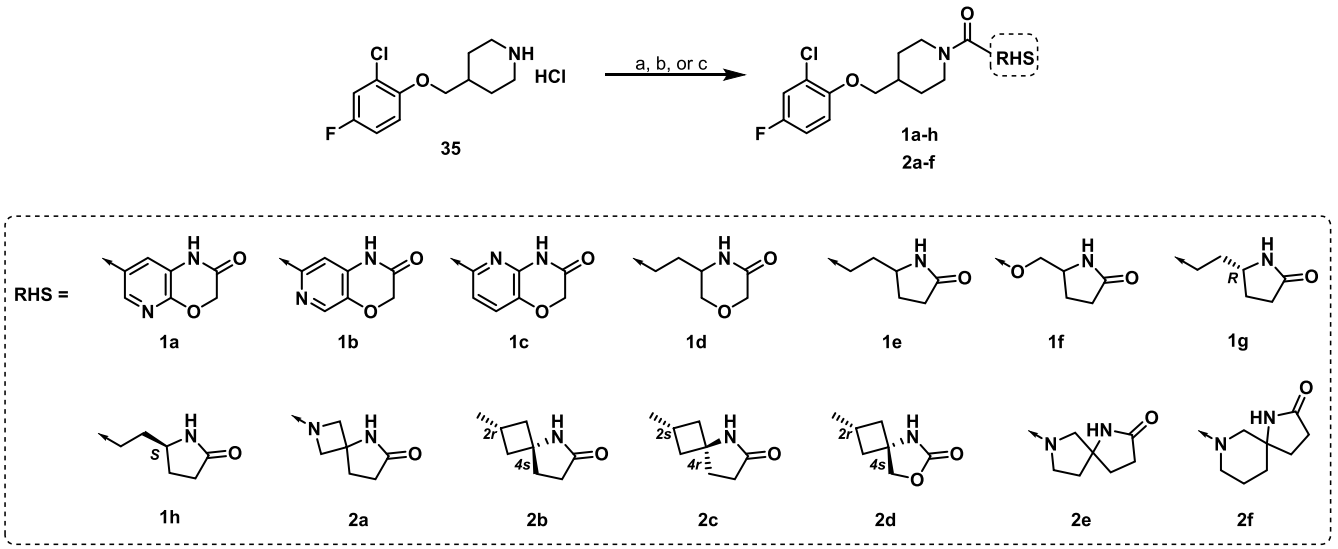


Figure 7. PD dose−response of **4f** in C57BL/6J mice (8W), 1 h after oral administration at 0.1−10 mg/kg, po. (A) 2-AG level in the mouse brain. (B) AA level in the mouse brain. Data are presented as the mean ± standard error of the mean (SEM) (n = 4); $^{\#}p < 0.025$ vs control by Williams test.

Scheme 1. Synthesis of 1a−h/2a−f in Table 1 or Table 2[a]



[a]Reagents and conditions: (a) (1) RHS acids, EDCI, HOBt, DIPEA, DMF, or MeCN, rt, 2 h—overnight, 46−96% for **1a−e**, **2b**, and **2d**, 56% in three steps from **19** for **2b**; (2) chiral separation by preparative SFC, 49% for **1g**, 48% for **1h**; (b) 5-(hydroxymethyl)pyrrolidin-2-one or amine **32**, 4-nitrophenyl chloroformate, Et_3N, THF, 0 °C to rt or 0 °C to reflux, overnight, 42% for **1f** and 5% for **2f**; (c) Amine **28** or 1,7-diazaspiro[4.4]nonan-2-one hydrochloride, triphosgene, DIPEA, MeCN, −10 °C to rt, 2.5−4 h, 26% for **2a** and 31% for **2e**.

CHEMISTRY

Scheme 1 shows the synthesis of compounds **1a−h** and **2a−f** in Table 1 or Table 2. Compounds **1a−e**, **2b**, and **2d** were synthesized by amidation of amine **35** with the corresponding RHS acids using EDCI and HOBt. Chiral compounds **1g** and **1h** were obtained by preparative chiral SFC of **1e**. Compounds **1f** and **2f** were synthesized via 4-nitrophenyl carbamate of **35** followed by addition of 5-(hydroxymethyl)-pyrrolidin-2-one or amine **32** in the presence of Et_3N. Compounds **2a** and **2e** were synthesized via the formation of a reactive carbamate of **35** with triphosgene and DIPEA, followed by addition of amine **28** or 1,7-diazaspiro[4.4]-nonan-2-one hydrochloride.

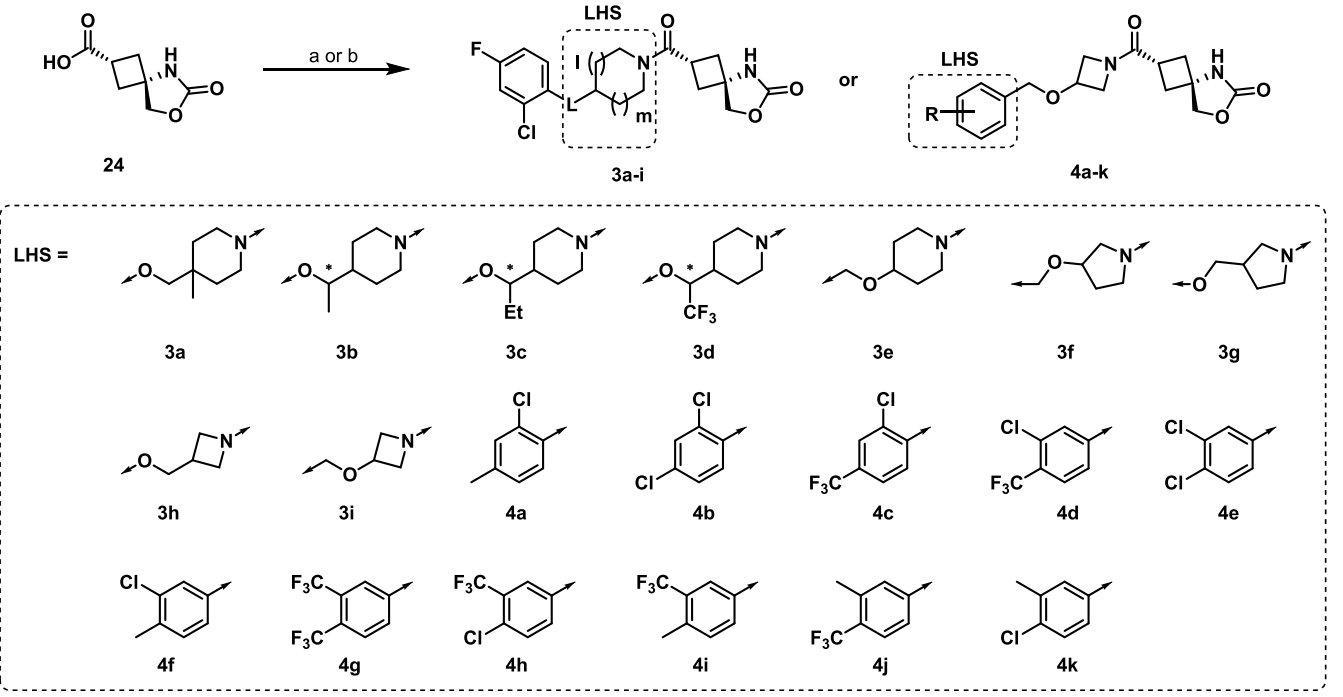
Scheme 2 shows the synthesis of compounds **3a−i** and **4a−k** in Table 3 or Table 4. Basically, they were synthesized by amidation of the corresponding LHS amines with RHS acid **24** using EDCI and HOBt. Chiral compounds **3b−d** were obtained by preparative chiral high-performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC).

Scheme 3 shows the synthesis of RHS acids **6** and **11** for compounds **1b** and **1d** in Table 1. Acid **6** was obtained via oxidation of the known aldehyde **5**.[36] The synthesis of **11** started from *N*-α-carbobenzoxy-DL-glutamic acid γ-*t*-butyl ester **7**. Reduction of the carboxylic acid in **7** by $NaBH_4$ via mixed acid anhydride gave alcohol **8**. The removal of the Cbz group afforded amino alcohol **9**, which was reacted with chloroacetyl chloride to form morpholinone **10**. Finally, deprotection of the *t*-butyl ester furnished acid **11**.

Scheme 4 shows the synthesis of RHS spirocyclobutane acids **18**, **21**, and **24** for the compounds in Tables 2 and 4. Starting from 3-oxocyclobutanecarboxylic acid derivatives **12a** (*t*-Bu ester) and **12b** (Et ester), the oxo groups were first transformed into nitro groups via a two-step sequence: (1)

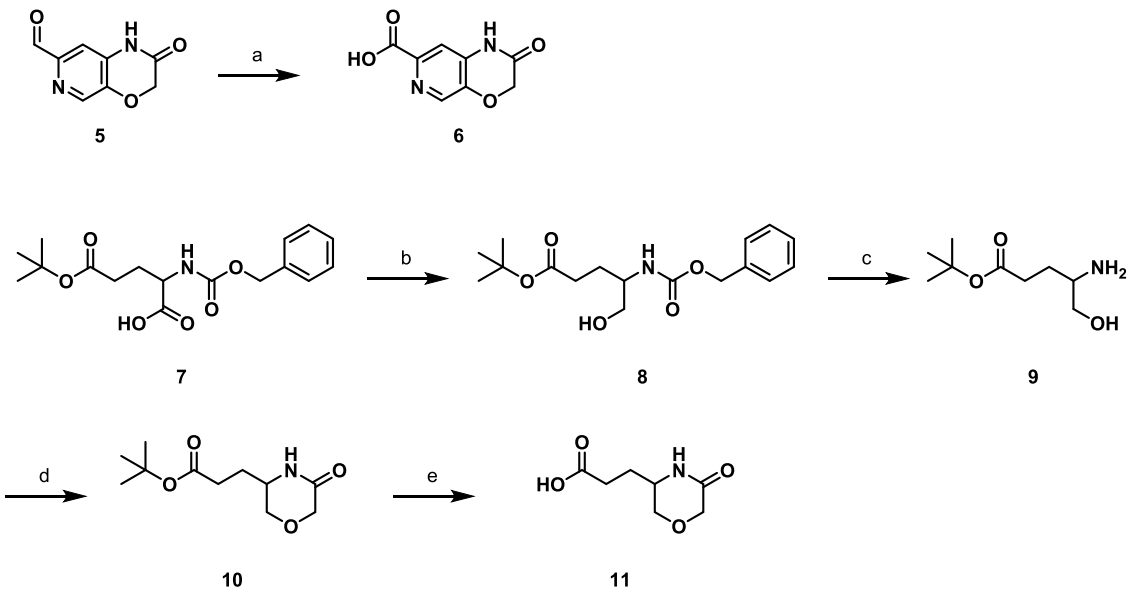
https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of 3a−i/4a−k in Table 3 or Table 4[a]



[a]Reagents and conditions: (a) Amine **41a**, EDCI, HOBt, DIPEA, DMF, rt, 4 h; (2) chiral separation by preparative HPLC, 25% for **3b** in three steps from **23b**; (b) (1) LHS amines, EDCI, HOBt, DIPEA, DMF, or MeCN, rt, 3−64 h, 32−89% for **3a**, **3e−i**, and **4a−k**; (2) chiral separation by preparative SFC or HPLC, 24% for **3c** and 17% for **3d** in two steps.

Scheme 3. Synthesis of RHS Acids 6 and 11[a]

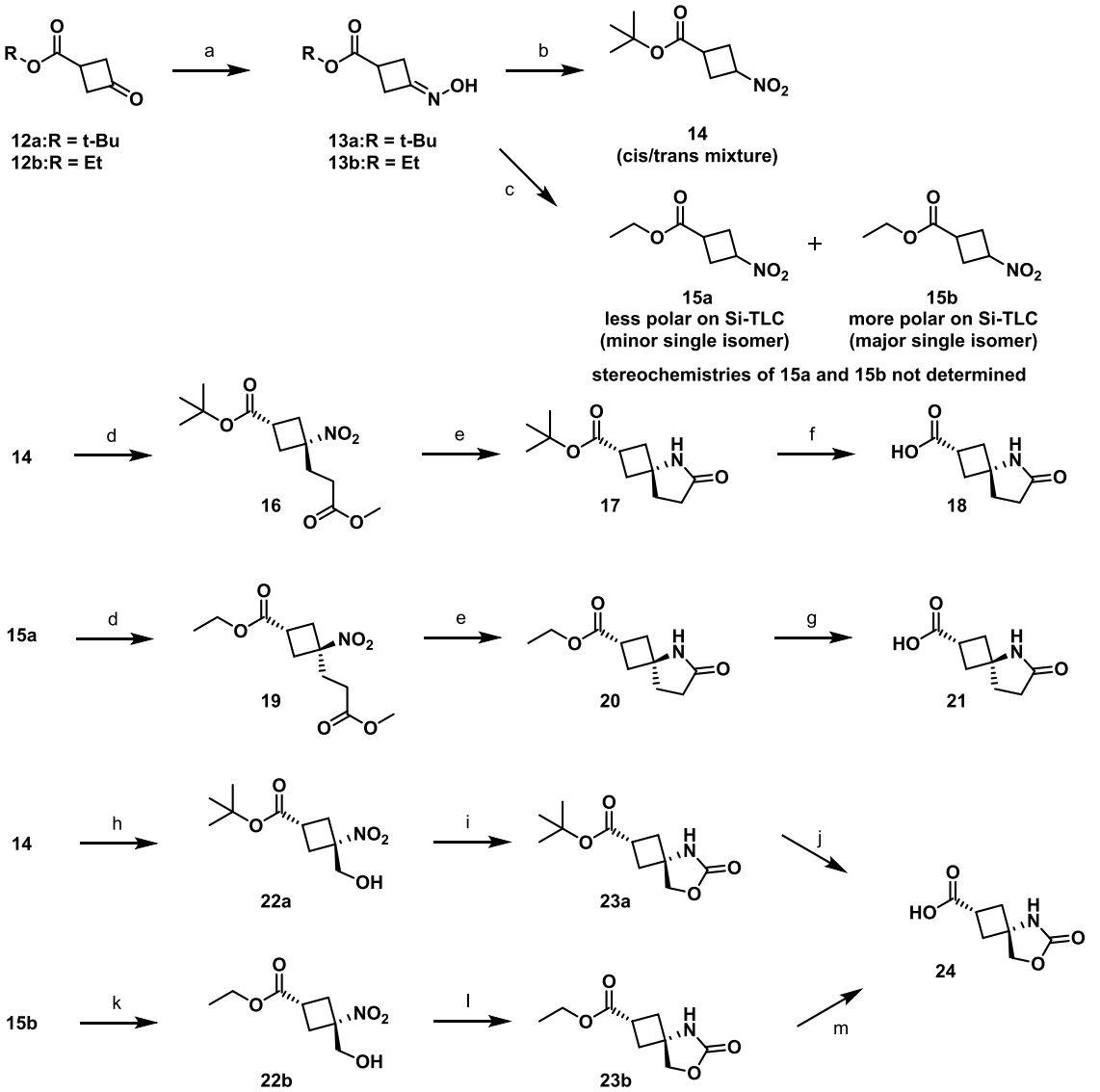


[a]Reagents and conditions: (a) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*-BuOH/H_2O, rt, 3 h, 68%; (b) isobutyl chloroformate, Et_3N, 0 °C, 0.5 h, then $NaBH_4$, 0 °C, 0.5 h, 88%; (c) H_2, 10% Pd-C, EtOAc, rt, 6 h, then H_2, 20% $Pd(OH)_2$-C, EtOAc, rt, 6 h, 100%; (d) chloroacetic chloride, Et_3N, THF, −78 °C, 0.5 h, then *t*-BuOK, −78 °C to rt, 2 h, 40%; (e) TFA, rt, overnight, 93%.

oxime formation with hydroxylamine, (2) oxidation with urea hydrogen peroxide (UHP) and trifluoroacetic anhydride (TFAA), affording nitro compounds **14**, **15a**, and **15b**. Compound **14** (*t*-Bu ester derivative) was used as a cis/trans mixture for the synthesis of target acids **18** and **24**. On the other side, Et ester derivatives **15a** (minor single isomer, stereochemistry not determined) and **15b** (major single isomer, stereochemistry not determined) could be separated by silica gel column chromatography and used in the next steps to synthesize **21** and **24**, respectively. In light of scale-up synthesis for **24**, *t*-Bu ester derivative **14** was preferentially utilized. The spirolactam structures in **18** and **21** were formed via a three-step sequence as follows: (1) Michael addition of **14** and **15a** to methyl acrylate with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), (2) reduction of the nitro groups in **16** and **19** with $NaBH_4/NiCl_2$ followed by

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of RHS Acids 18, 21, and 24[a]



[a]Reagents and conditions: (a) NH_2OH-HCl, NaOAc, NaH_2PO_4, EtOH, reflux, 4−18 h, 92% for **13a** and 89% for **13b**; (b) UHP, Na_2HPO_4, TFAA, MeCN, −10 °C to 80 °C, 2.5 h, 64%; (c) UHP, Na_2HPO_4, TFAA, MeCN, 80 °C, 2 h, 18% for **15a** and 25% for **15b**; (d) methyl acrylate, DBU, 0 °C, 20 min to 1 h, 30% for **16** and 25% for **19**; (e) $NaBH_4$, $NiCl_2$·$6H_2O$, then aq. K_2CO_3, −10 °C to rt, 3−7 h, 78% for **17**; (f) TFA, 0 °C to rt, 4 h, 86%; (g) 2 M NaOH, THF/MeOH/H_2O, rt, overnight; (h) 37% aq. formaldehyde, Et_3N, MeCN, 0 °C to rt, 2 h, 52%; (i) (1) H_2 (50 bar), Raney Ni, isopropanol, 70 °C, 3 h, 82%, (2) triphosgene, Et_3N, THF, −10 °C to rt, 2.5 h, 81%; (j) TFA, 0 °C to rt, 2 h, 92%; (k) 35% aq. formaldehyde, Et_3N, MeCN, rt, 1 h, 47%; (l) (1) H_2, 10% Pd-C, MeOH/THF, rt, 72 h (2) triphosgene, DIPEA, THF, −10 °C, 0.5 h, 51% in two steps; (m) 2 M NaOH, THF/MeOH, rt, 1 h.

intramolecular cyclization with K_2CO_3, and (3) cleavage of the ester groups in **17** and **20**. On the other side, the spiro carbamate structure was formed via a four-step sequence as follows: (1) Henry reaction of **14** and **15b** to formaldehyde, (2) reduction of the nitro groups in **22a** and **22b** under hydrogen atmosphere in the presence of Raney Ni or Pd-C, (3) reaction of the corresponding aminoalcohols with triphosgene, (4) cleavage of the *t*-Bu ester groups in **23a** and **23b**. It should be noted that both the Michael addition and the Henry reaction gave the stereoisomers of **16**, **19**, **22a**, and **22b** which were easily separable by silica gel column chromatography, respectively.

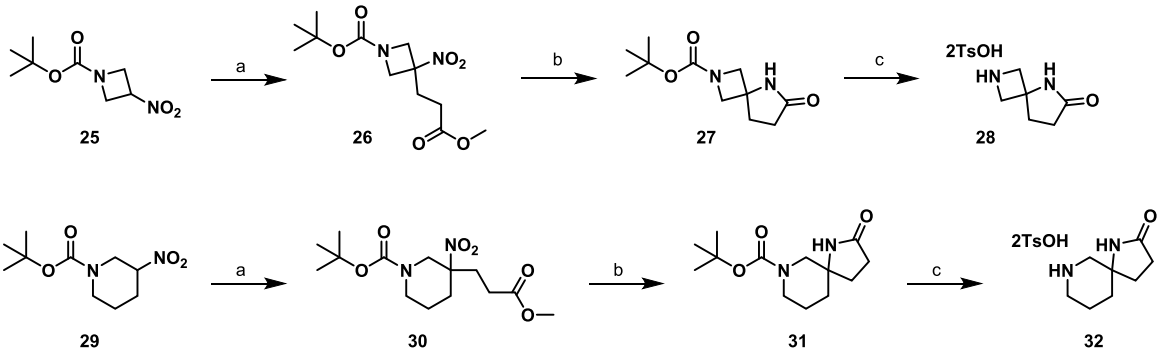
Scheme 5 shows the synthesis of RHS spiroamines **28** and **32** for the compounds in Table 2. They were synthesized from Boc-3-nitroazetidine **25** and Boc-3-nitropiperidine **29** via similar three-sequence: (1) Michael addition of **25** and **29** to methyl acrylate, (2) reduction of the nitro groups in **26** and **30** with $NaBH_4$/$NiCl_2$ followed by intramolecular cyclization with K_2CO_3, (3) cleavage of the Boc groups in **27** and **31**.

Scheme 6 shows the synthesis of LHS amine **35** as the common structure for the compounds in Tables 1 and 2. Mitsunobu reaction of Boc-4-(hydroxymethyl)piperidine **33** followed by deprotection of Boc group gave **35**.

Scheme 7 shows the synthesis of LHS amines **38**, **41a−e**, and **44a−c** with various linkers for the compounds in Table 3. Nucleophilic aromatic substitution of 1-chloro-2,5-difluorobenzene by Boc-4-(hydroxymethyl)-4-methylpiperidine **36** occurred at 2-position in the presence of t-BuOK, followed by deprotection of Boc group, furnished **38**. Compounds

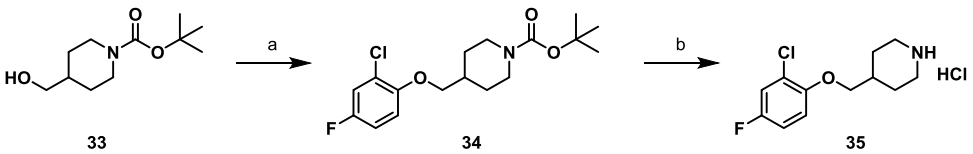
https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 5. Synthesis of RHS Amines 28 and 32[a]



[a]Reagents and conditions: (a) methyl acrylate, K_2CO_3, MeOH, 0 °C or 0 °C to rt, 3−4 h, 53% for **26** and 62% for **30**; (b) $NaBH_4$, $NiCl_2·6H_2O$, then aq. K_2CO_3, −10 °C to rt, 3.5−4 h, 76% for **27** and 81% for **31**; (c) *p*-TsOH-H_2O, EtOAc, reflux, 3−18 h, 90% for **28** and 99% for **32**.

Scheme 6. Synthesis of LHS Amine 35[a]



[a]Reagents and conditions: (a) 2-chloro-4-fluorophenol, ADDP, PBu_3, 0 °C to rt, 18 h, THF, 95%; (b) 4 M HCl/EtOAc, rt, 14 h, 67%.

41a−e with phenyloxymethylene/phenyloxymethine-type linkers were synthesized from alcohols **39a−e** via a three-step sequence: (1) sulfonylation by MsCl, Tf_2O, or TsCl with Et_3N or pyridine, (2) nucleophilic replacement by 2-chloro-4-fluorophenol with Cs_2CO_3, K_3PO_4, or K_2CO_3, (3) cleavage of the Boc groups in **40a−e** with *p*-TsOH-H_2O or 4 M HCl in EtOAc. On the other side, compounds **44a−c** with phenylmethyleneoxy-type linkers were synthesized from secondary alcohols **42a−c** via a two-step sequence: (1) alkylation with 1-(bromomethyl)-2-chloro-4-fluorobenzene with NaH, (2) cleavage of the Boc groups in **43a−c** with *p*-TsOH-H_2O.

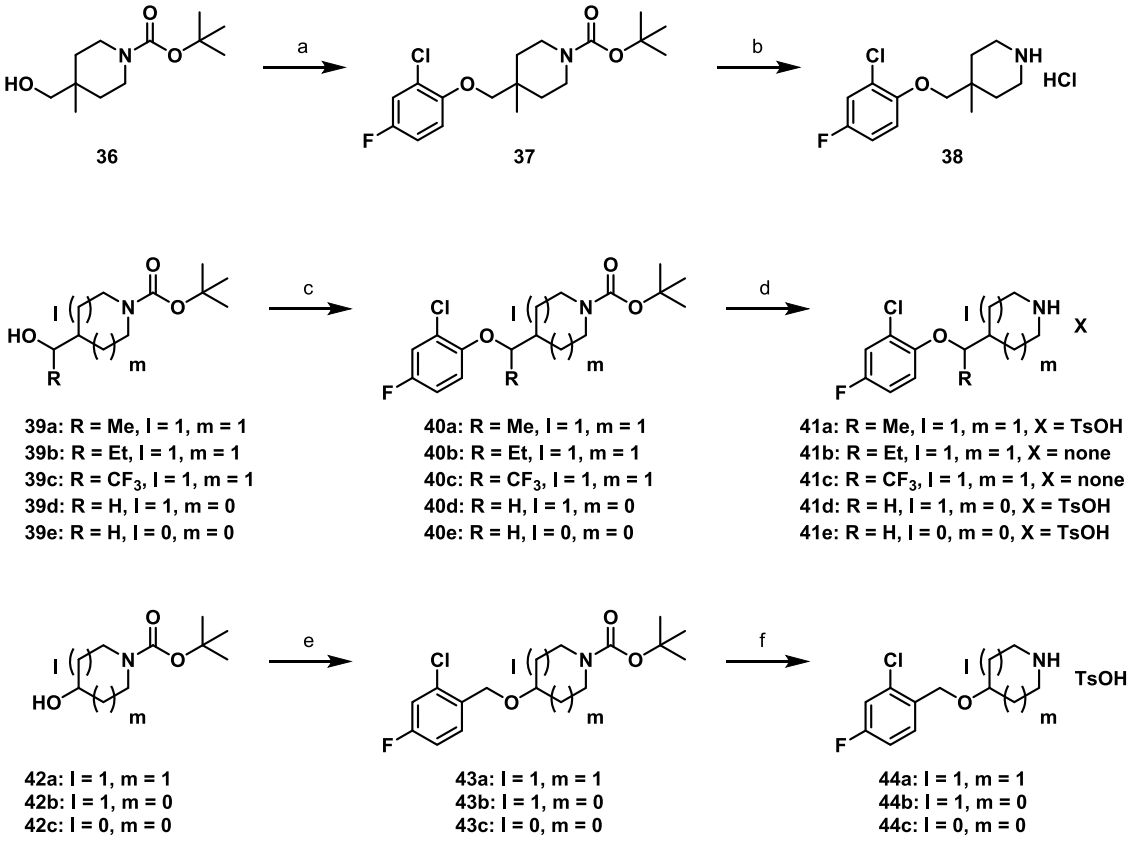
Scheme 8 shows the synthesis of LHS amines **46a−g**, **48a−c**, and **50** for the compounds in Table 4. Compounds **46a−d** were synthesized from alcohols **45a−d** via a three-step sequence: (1) sulfonylation with MsCl and Et_3N, (2) nucleophilic displacement by *t*-butyl-3-hydroxyazetidine-1-carboxylate with NaH, and (3) cleavage of Boc group with *p*-TsOH-H_2O. Compounds **46e,f** were synthesized from alcohols **45e,f** via a three-step sequence: (1) bromination with PBr_3, (2) nucleophilic displacement by *t*-butyl-3-hydroxyazetidine-1-carboxylate with NaH, and (3) cleavage of Boc group with *p*-TsOH-H_2O. Compound **46g** was synthesized from alcohol **45b** via four-step sequence: (1) sulfonylation with MsCl and Et_3N, (2) nucleophilic displacement by *t*-butyl-3-hydroxyazetidine-1-carboxylate with NaH, (3) methylation by Suzuki coupling, (4) cleavage of Boc group with *p*-TsOH-H_2O. Compounds **48a−c** were synthesized from chlorides **47a−c** via a two-step sequence: (1) nucleophilic displacement by *t*-butyl-3-hydroxyazetidine-1-carboxylate with NaH, (2) cleavage of Boc group with *p*-TsOH-H_2O. Compound **50** was synthesized from acid **49** via four-step sequence: (1) reduction of the carboxylic acid by BH_3−THF complex, (2) sulfonylation with MsCl and Et_3N, (3) nucleophilic displacement by *t*-butyl-3-hydroxyazetidine-1-carboxylate with NaH, (4) cleavage of Boc group with *p*-TsOH-H_2O.

CONCLUSIONS

To generate a novel, potent, and reversible MAGL inhibitor, the SBDD approach as well as monitoring of LLE values were utilized for lead generation/optimization process. The co-crystal structure of hit compound **1** (MAGL IC_{50} = 15 nM, LLE = 4.5) with MAGL revealed the unique polar interactions with the side chains of Arg57 and His121 by the amide group of 3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl moiety. While maintaining these key interactions, bioisosteric transformation of 3-oxo-3,4-dihydro-2*H*-benzo-[*b*][1,4]oxazin-6-yl moiety based on SBDD led to the discovery of novel spiro chemical series (azetidine-lactam **2a**, cyclobutane-lactam **2b**, and cyclobutane-carbamate **2d**) with higher LLE values than that of hit **1** (5.3, 5.4, and 5.9, respectively). To improve the hERG liability of the lead compound **2d**, optimization was conducted for the remaining molecular portion, 4-((2-chloro-4-fluorophenoxy)methyl) piperidine moiety. Modification of the ring size of the amine portion, the oxymethylene linker, and the substituents on the benzene ring enabled us to identify (2*s*,4*s*)-2-((3-((3-chloro-4-methylbenzyl)oxy)azetidin-1-yl)carbonyl)-7-oxa-5-azaspiro-[3.4]octan-6-one (**4f**), which showed potent activity (MAGL IC_{50} = 6.2 nM), a high LLE (5.9), good selectivity toward the closely related serine hydrolases (FAAH IC_{50} > 10000 nM and ABHD6 IC_{50} > 10000 nM), no significant binding potentials to cannabinoid receptors (CB_1: 19% and CB_2: 5% at 10 μM), and low hERG liability (14.4% inh. at 10 μM, manual patch clamp, without BSA). In vivo profiling of **4f** in mice showed good pharmacokinetics, BBB penetration, and significant pharmacodynamic changes (2-AG increase and AA decrease) at 0.3−10 mg/kg, po. that were consistent with the dose-dependent brain concentrations of **4f** in the brain, indicating that **4f** could be a promising reversible MAGL inhibitory agent. Further detailed pharmacological characterization of **4f** will be reported in due course.

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 7. Synthesis of LHS Amines 38, 41a−e, and 44a−c[a]



[a]Reagents and conditions: (a) 1-chloro-2,5-difluorobenzene, *t*-BuOK, THF, 0 °C to rt, overnight, then reflux, 4 h, 57%; (b) 2 M HCl/MeOH, rt, overnight, 92%; (c) (1) MsCl, Et_3N, THF/MeCN, 0 °C to rt, 3−6 days, toward **40a,b**; Tf_2O, pyridine, 0 °C to rt, overnight, toward **40c**; TsCl, pyridine, 0 °C to rt, overnight, toward **40d,e**, 54-97%; (2) 2-chloro-4-fluorophenol, Cs_2CO_3, DMF, 80 °C, overnight, for **40a−c**; 2-chloro-4-fluorophenol, K_3PO_4, DMF, 80 °C, 2 h, for **40d**; 2-chloro-4-fluorophenol, K_2CO_3, DMF, 80 °C, overnight, for **40e**, 48−87% for **40a** and **40d,e**; (d) *p*-TsOH-H_2O, EtOAc, 70 °C to reflux, 2 h, 73−92% for **41a**, and **41d,e**; 4 M HCl/EtOAc, rt, 2 h, 43% for **41b** and 16% for **41c** in two steps; (e) 1-(bromomethyl)-2-chloro-4-fluorobenzene, NaH, DMF, 0 °C to rt, 16 h, 78−100% for **43a−c**; (f) *p*-TsOH-H_2O, EtOAc, reflux, 3 h, 25−76% for **44a−c**.

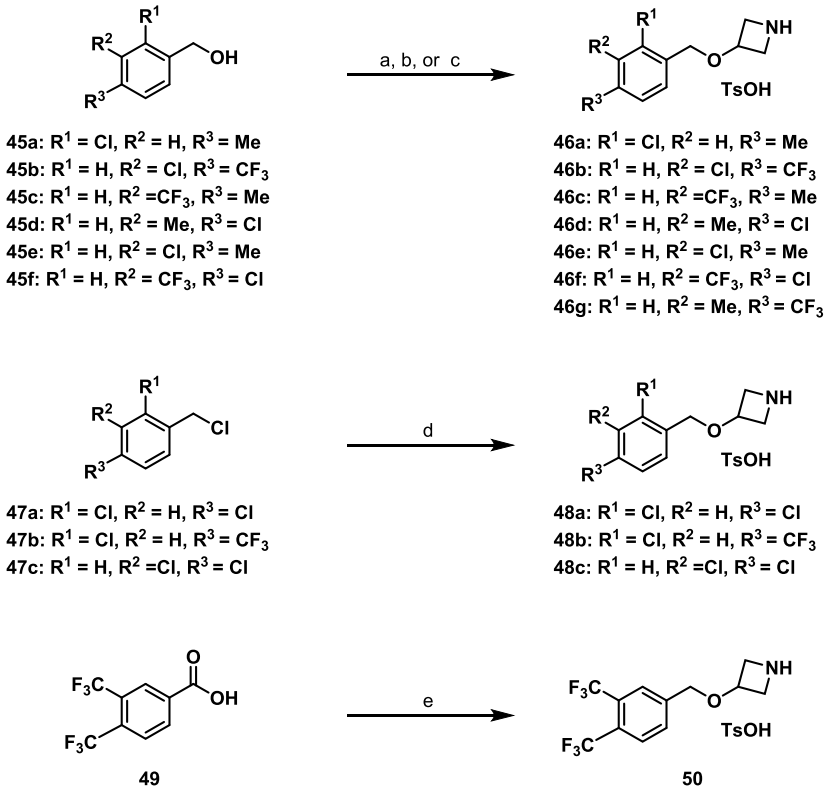
EXPERIMENTAL SECTION

General Chemistry Information. All solvents and reagents were obtained from commercial suppliers and used without further purification. Melting points were determined using a DSC1 system (Mettler-Toledo International Inc., Greifensee, Switzerland). Proton and carbon nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on Bruker (300, 400, or 500 MHz) instruments. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent, and coupling constants (J) are in hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, and brs = broad signal), and coupling constants. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Chromatographic purification was performed on silica gel columns [(Merck Kieselgel 60, 70−230 mesh size or 230−400 mesh size, Merck) or (Chromatorex NH-DM 1020, 100−200 mesh size)] or on Purif-Pack (SI or NH, SHOKO SCIENTIFIC). LCMS analysis was performed on Shimadzu UFLC/MS (Prominence UFLC high-pressure gradient system/LCMS-2020) or Agilent LC/MS system (Agilent 1200SL/Agilent 6130MS), operating in ESI (+ or −) or APCI (+ or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% TFA containing water/acetonitrile or 5 mM ammonium acetate containing water/acetonitrile mobile phase and detected at 220 nm. Analytical HPLC was performed with a Corona Charged Aerosol Detector (CAD), a Nano quantity analyte detector (NQAD), or a photodiode array detector. The column was a Capcell Pak C18AQ (50 mm × 3.0 mm I.D., Shiseido, Japan) or L-column 2 ODS (30 mm × 2.0 mm I.D., CERI, Japan) with a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phases A and B under a neutral condition were a mixture of 50 mmol/L ammonium acetate, water, and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L ammonium acetate and acetonitrile (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5% to 95% over 3 min, 95% over the next 1 min. Mobile phases A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mmol/L ammonium formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min. The purities of all tested compounds were >95% as determined by elemental analyses within ±0.4% of the calculated values or analytical HPLC. Yields were not optimized.

*7-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-1H-pyrido[2,3-b][1,4]oxazin-2(3H)-one (**1a**).* A mixture of **35** (43.3 mg, 0.15 mmol), 2-oxo-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]oxazine-7-carboxylic acid (30 mg, 0.15 mmol), HOBt-H_2O (28.4 mg, 0.19 mmol), EDCI (35.5 mg, 0.19 mmol), DIPEA (0.067 mL, 0.39 mmol), and DMF (2 mL) was stirred at rt for 3 h. After addition of water, the resultant precipitates were collected, washed with water and EtOAc, dried in vacuo, and triturated with hot EtOAc to give **1a** (48 mg, 0.114 mmol, 74%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (2H, qd, J = 12.2, 4.0 Hz), 1.73−2.20 (3H, m), 2.74−3.22 (2H, m), 3.61−4.02 (3H, m), 4.30−4.61 (1H, m), 4.83 (2H, s), 7.14−7.25 (3H, m), 7.39−7.47 (1H, m), 7.83 (1H, d,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 8. Synthesis of LHS amines 46a–g, 48a–c, and 50[a]



[a]Reagents and conditions: (a) (1) MsCl, Et_3N, THF, 0 °C to rt, 0.5–16 h, quant.; (2) *t*-butyl 3-hydroxyazetidine-1-carboxylate, NaH, DMF, 0 °C to rt, 3–38 h, 87–93%; (3) *p*-TsOH-H_2O, EtOAc, reflux, 2 h, 69–88% for **46a–d**; (b) (1) PBr_3, DME, 0 °C to rt, 2.5–3 h, 86-100%; (2) *t*-butyl 3-hydroxyazetidine-1-carboxylate, NaH, DMF, 0 °C to rt, overnight, 89–99%; (3) *p*-TsOH-H_2O, EtOAc, reflux, 2–3 h, 83-92% for **46e,f**; (c) (1) MsCl, Et_3N, THF, 0 °C to rt, 100%; (2) *t*-butyl 3-hydroxyazetidine-1-carboxylate, NaH, DMF, 0 °C to rt, 87%; (3) trimethylboroxine, $Pd(OAc)_2$, SPhos, K_3PO_4, toluene–H_2O, 100 °C, 16 h, 63%; (4) *p*-TsOH-H_2O, EtOAc, reflux, 2 h, 74% for **46 g**; (d) (1) *t*-butyl 3-hydroxyazetidine-1-carboxylate, NaH, DMF, 0 °C to rt, overnight, 79–94%; (2) *p*-TsOH-H_2O, EtOAc, reflux, 2–3 h, 84-86% for **48a–c**; (e) (1) BH_3–THF complex, THF, 0 °C to rt, 18 h, 100%; (2) MsCl, Et_3N, THF, 0 °C to rt, 2 h, quant.; (3) *t*-butyl 3-hydroxyazetidine-1-carboxylate, NaH, DMF, 0 °C to rt, 64 h, 76%; (4) *p*-TsOH-H_2O, EtOAc, reflux, 2 h, 93% for **50**.

J = 2.1 Hz), 10.92 (1H, brs). ^{13}C NMR (101 MHz, DMSO-d_6) δ 35.8, 67.5, 73.6, 115.0, 115.19, 115.22, 115.3, 117.4, 117.7, 122.4, 122.5, 127.5, 139.3, 151.19, 151.22, 151.6, 155.0, 157.3, 164.3, 166.6. LCMS (ESI/APCI) *m*/*z* 420.1 $[M + H]^+$. Anal. Calcd for $C_{20}H_{19}ClFN_3O_4$: C, 57.22; H, 4.56; N, 10.01. Found: C, 57.26; H, 4.77; N, 10.02. HPLC purity 99.2%. Mp 237 °C.

*7-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-1H-pyrido[3,4-b][1,4]oxazin-2(3H)-one (**1b**).* A mixture of **35** (70 mg, 0.25 mmol), **6** (48.5 mg, 0.25 mmol), HOBt (40.5 mg, 0.30 mmol), EDCI (57.5 mg, 0.30 mmol), DIPEA (0.104 mL, 0.60 mmol), and DMF (1.5 mL) was stirred at rt for 16 h. After addition of water, the resultant precipitate was collected, washed with water, and dried in vacuo. The residual solid was triturated with hot EtOAc/IPA to give **1b** (67.0 mg, 0.160 mmol, 64%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22–1.41 (2H, m), 1.69–1.97 (2H, m), 2.00–2.20 (1H, m), 2.75–2.89 (1H, m), 2.99–3.16 (1H, m), 3.86–4.00 (3H, m), 4.42–4.55 (1H, m), 4.74 (2H, s), 7.06 (1H, s), 7.14–7.21 (2H, m), 7.38–7.47 (1H, m), 8.15 (1H, s), 11.19 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.6, 29.4, 35.9, 42.0, 46.8, 67.3, 73.7, 110.6, 114.9, 115.15, 115.21, 115.3, 117.4, 117.6, 122.4, 122.5, 135.1, 136.1, 140.8, 149.2, 151.21, 151.24, 155.0, 157.3, 165.2, 166.5. LCMS (ESI/APCI) *m*/*z* 420.1 $[M + H]^+$. Anal. Calcd for $C_{20}H_{19}ClFN_3O_4$: C, 57.22; H, 4.56; N, 10.01. Found: C, 57.33; H, 4.73; N, 9.94. HPLC purity 100%. Mp 226 °C.

*6-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (**1c**).* To a stirred mixture of **35** (130 mg, 0.46 mmol), 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]-oxazine-6-carboxylic acid (90 mg, 0.46 mmol), EDCI (107 mg, 0.56 mmol), HOBt-H_2O (71.1 mg, 0.46 mmol), and MeCN (4.00 mL) was added Et_3N (0.155 mL, 1.11 mmol) at rt, and the mixture was stirred at rt overnight. The mixture was diluted with aq. $NaHCO_3$ and extracted with MeOH/EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, passed through a short pad of silica gel eluting with MeOH/EtOAc (1:20), and concentrated in vacuo. The residue was triturated with EtOAc/IPE to give **1c** (105 mg, 0.250 mmol, 54%) as a pale brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22–1.41 (2H, m), 1.82 (2H, brs), 2.08 (1H, d, *J* = 5.7 Hz), 2.74–3.24 (2H, m), 3.56–3.83 (1H, m), 3.94 (2H, d, *J* = 6.4 Hz), 4.26–4.58 (1H, m), 4.83 (2H, s), 7.15–7.17 (1H, m), 7.19 (1H, d, *J* = 1.9 Hz), 7.22 (1H, d, *J* = 2.3 Hz), 7.43 (1H, dt, *J* = 8.1, 1.6 Hz), 7.83 (1H, d, *J* = 2.3 Hz), 10.94 (1H, brs). ^{13}C NMR (101 MHz, DMSO-d_6) δ 35.8, 67.5, 73.6, 114.9, 115.17, 115.20, 115.3, 117.4, 117.7, 122.39, 122.41, 122.5, 127.5, 139.3, 151.19, 151.22, 151.6, 155.0, 157.3, 164.3, 166.6. LCMS (ESI/APCI) *m*/*z* 420.2 $[M + H]^+$. Anal. Calcd for $C_{20}H_{19}ClFN_3O_4$: C, 57.22; H, 4.56; N, 10.01. Found: C, 57.30; H, 4.82; N, 9.86. HPLC purity 100%. Mp 237 °C.

*5-(3-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidin-1-yl)-3-oxopropyl)morpholin-3-one (**1d**).* A mixture of **35** (146 mg, 0.52 mmol), **11** (90 mg, 0.52 mmol), EDCI (120 mg, 0.62 mmol), HOBt-H_2O (80 mg, 0.52 mmol), Et_3N (0.173 mL, 1.25 mmol), and MeCN (4.00 mL) was stirred at rt overnight. The mixture was diluted with aq. $NaHCO_3$ and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with MeOH/EtOAc (0–9%) to give **1d** (200 mg, 0.501 mmol, 96%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27–1.46 (2H, m), 1.82–1.95 (3H, m), 1.96–2.24

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX–XXX

(2H, m), 2.47 (2H, t, J = 7.0 Hz), 2.54−2.74 (1H, m), 3.10 (1H, td, J = 13.1, 2.4 Hz), 3.46−3.58 (1H, m), 3.62 (1H, brs), 3.74−3.99 (4H, m), 4.05−4.26 (2H, m), 4.69 (1H, d, J = 13.6 Hz), 6.74 (1H, brs), 6.80−7.00 (2H, m), 7.13 (1H, dd, J = 8.3, 3.0 Hz). LCMS (ESI/APCI) m/z 399.1 $[M + H]^+$. HPLC purity 100%.

*5-(3-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidin-1-yl)-3-oxopropyl)pyrrolidin-2-one (**1e**).* A mixture of **35** (143 mg, 0.51 mmol), 3-(5-oxopyrrolidin-2-yl)propanoic acid (80 mg, 0.51 mmol), HOBt-H_2O (94 mg, 0.61 mmol), EDCI (117 mg, 0.61 mmol), DIPEA (0.222 mL, 1.27 mmol), and DMF (2 mL) was stirred at rt for 3 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 0−100% EtOAc in hexane; 5−30% MeOH in EtOAc) to give **1e** (174 mg, 0.454 mmol, 89%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22−1.43 (2H, m), 1.70−1.94 (4H, m), 1.96−2.46 (7H, m), 2.54−2.72 (1H, m), 3.01−3.17 (1H, m), 3.62−3.99 (4H, m), 4.59−4.76 (1H, m), 6.22 (1H, brs), 6.79−6.97 (2H, m), 7.12 (1H, dd, J = 8.0, 2.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 27.3, 28.4, 29.6, 30.3, 31.9, 36.3, 41.6, 45.4, 54.2, 73.8, 113.9, 114.1, 114.15, 114.23, 117.4, 117.7, 123.6, 123.7, 150.96, 150.99, 155.3, 157.7, 170.36, 170.38, 178.3. LCMS (ESI/APCI) m/z 383.2 $[M + H]^+$. Anal. Calcd for $C_{19}H_{24}ClFN_2O_3$: C, 59.61; H, 6.32; N, 7.32. Found: C, 59.55; H, 6.41; N, 7.29. HPLC purity 96.2%. Mp 103 °C.

*(5-Oxopyrrolidin-2-yl)methyl 4-((2-chloro-4-fluorophenoxy)-methyl)piperidine-1-carboxylate (**1f**).* To a stirred mixture of 5-(hydroxymethyl)pyrrolidin-2-one (101 mg, 0.88 mmol), Et_3N (0.244 mL, 1.75 mmol), and THF (4.00 mL) was added 4-nitrophenyl chloroformate (186 mg, 0.92 mmol) at 0 °C, and the mixture was stirred at rt overnight. To this mixture were added Et_3N (0.366 mL, 2.63 mmol) and **35** (246 mg, 0.88 mmol) at rt, and the mixture was stirred overnight. The mixture was diluted with water and extracted with EtOAc. The extract was washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with MeOH/EtOAc (0−20%), and crystallized from EtOAc/IPE to give a crude material. This material (171 mg) was purified by preparative HPLC and the target fractions were concentrated in vacuo. The residue was passed through the short pad of NH silica gel eluting with MeOH/EtOAc (10%). The filtrate was concentrated in vacuo, and the residue was crystallized from EtOAc/IPE to give **1f** (141 mg, 0.366 mmol, 42%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09−1.34 (2H, m), 1.66−1.86 (3H, m), 1.91−2.05 (1H, m), 2.05−2.24 (3H, m), 2.81 (2H, brs), 3.68−3.79 (1H, m), 3.82−4.14 (6H, m), 7.17 (2H, dd, J = 6.6, 1.7 Hz), 7.42 (1H, dt, J = 8.4, 1.6 Hz), 7.85 (1H, s). LCMS (ESI/APCI) m/z 385.1 $[M + H]^+$. Anal. Calcd for $C_{18}H_{22}ClFN_2O_4$: C, 56.18; H, 5.76; N, 7.28. Found: C, 56.09; H, 5.72; N, 7.21. HPLC purity 100%.

*(5R)-5-(3-{4-[(2-chloro-4-fluorophenoxy)methyl]piperidin-1-yl}-3-oxopropyl)pyrrolidin-2-one (**1g**) and (5S)-5-(3-{4-[(2-chloro-4-fluorophenoxy)methyl]piperidin-1-yl}-3-oxopropyl)pyrrolidin-2-one (**1h**).* Racemate **1e** (1.26 g, 3.29 mmol) was optically resolved by preparative SFC (CHIRALPAK IA, 20 mm I.D. × 250 mm L., 5 μm particle size, Daicel, Japan, eluent: CO_2/MeOH = 600:400 v/v, flow rate: 55 mL/min, temp.: 35 °C) to give **1g** (0.611 g, 1.60 mmol, 49%) and **1h** (0.609 g, 1.59 mmol, 48%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21−1.45 (2H, m), 1.72−1.81 (1H, m), 1.82−1.94 (3H, m), 2.01 (1H, d, J = 13.2 Hz), 2.06−2.19 (1H, m), 2.21−2.37 (3H, m), 2.38−2.47 (2H, m), 2.63 (1H, t, J = 12.2 Hz), 3.09 (1H, td, J = 13.1, 2.4 Hz), 3.64−4.03 (4H, m), 4.69 (1H, d, J = 13.6 Hz), 6.36 (1H, brs), 6.80−6.87 (1H, m), 6.88−6.97 (1H, m), 7.13 (1H, dd, J = 8.1, 2.8 Hz). LCMS (ESI/APCI) m/z 383.1 $[M + H]^+$. HPLC purity 99.8% for **1g**, 99.7% for **1h**.

*2-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-2,5-diazaspiro[3.4]octan-6-one (**2a**).* To a cooled (−10 °C) mixture of **35** (80 mg, 0.29 mmol) and MeCN (4 mL) were added DIPEA (0.298 mL, 1.71 mmol) and Bis(trichloromethyl)-carbonate (29.7 mg, 0.10 mmol). The mixture was stirred at the same temperature for 2 h. To the mixture was added **28** (134 mg, 0.29 mmol), and the mixture was stirred at rt for 2 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residual solid was recrystallized from EtOAc-IPA to give **2a** (29 mg, 0.073 mmol, 26%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.29 (2H, m), 1.70−1.81 (2H, m), 1.88−2.05 (1H, m), 2.11−2.33 (4H, m), 2.67−2.85 (2H, m), 3.73−3.84 (2H, m), 3.86−3.96 (6H, m), 7.13−7.20 (2H, m), 7.38−7.46 (1H, m), 8.19 (1H, s). LCMS (ESI/APCI) m/z 396.1 $[M + H]^+$. Anal. Calcd for $C_{19}H_{23}ClFN_3O_3$: C, 57.65; H, 5.86; N, 10.62. Found: C, 57.29; H, 6.03; N, 10.50. HPLC purity 98.9%. Mp 197 °C.

*(2r,4s)-2-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-5-azaspiro[3.4]octan-6-one (**2b**).* A mixture of **18** (347 mg, 2.05 mmol), **35** (575 mg, 2.05 mmol), HOBt-H_2O (377 mg, 2.46 mmol), EDCI (472 mg, 2.46 mmol), DIPEA (1.79 mL, 10.3 mmol), and DMF (12 mL) was stirred at rt for 2 h. The mixture was diluted with water and extracted with EtOAc/IPA. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, filtered through a NH silica gel pad, and concentrated in vacuo. The residual solid was triturated with hot EtOAc/IPA to give **2b** (373 mg, 0.945 mmol, 46%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04−1.29 (2H, m), 1.69−1.89 (2H, m), 1.94−2.37 (9H, m), 2.52−2.66 (1H, m), 2.89−3.13 (2H, m), 3.75−3.99 (3H, m), 4.31−4.47 (1H, m), 7.11−7.22 (2H, m), 7.36−7.46 (1H, m), 7.93 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.6, 29.5, 30.5, 34.1, 36.0, 39.0, 41.5, 44.7, 55.9, 73.6, 114.9, 115.15, 115.23, 117.4, 117.6, 122.4, 122.5, 151.21, 151.24, 154.9, 157.3, 171.6, 176.0. LCMS (ESI/APCI) m/z 395.1 $[M + H]^+$. Anal. Calcd for $C_{20}H_{24}ClFN_2O_3$ 0.3H_2O: C, 60.01; H, 6.19; N, 7.00. Found: C, 60.07; H, 6.03; N, 7.00. HPLC purity 96.8%. Mp 196 °C.

*(2s,4r)-2-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-5-azaspiro[3.4]octan-6-one (**2c**).* A mixture of **21** (127 mg, 0.75 mmol), **35** (210 mg, 0.75 mmol), HOBt-H_2O (138 mg, 0.90 mmol), EDCI (173 mg, 0.90 mmol), DIPEA (0.654 mL, 3.75 mmol), and DMF (10 mL) was stirred at rt for 2 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, filtered through a NH silica gel pad, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 0−100% EtOAc in hexane; 3−25% MeOH in EtOAc) and recrystallized from EtOAc/IPA/heptane to give **2c** (165 mg, 0.418 mmol, 56%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06−1.31 (2H, m), 1.71−1.86 (2H, m), 1.91−2.15 (5H, m), 2.25−2.40 (4H, m), 2.54−2.67 (1H, m), 2.91−3.07 (1H, m), 3.12−3.26 (1H, m), 3.60−3.73 (1H, m), 3.83−3.99 (2H, m), 4.34−4.48 (1H, m), 7.12−7.21 (2H, m), 7.38−7.48 (1H, m), 8.12 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.5, 29.2, 29.3, 30.5, 35.2, 36.0, 38.7, 38.7, 41.5, 44.7, 57.9, 73.6, 114.9, 115.1, 115.16, 115.22, 117.4, 117.6, 122.3, 122.5, 151.21, 151.23, 154.9, 157.3, 171.9, 176.0. LCMS (ESI/APCI) m/z 395.1 $[M + H]^+$. Anal. Calcd for $C_{20}H_{24}ClFN_2O_3$: C, 60.83; H, 6.13; N, 7.09. Found: C, 61.01; H, 6.22; N, 7.08. HPLC purity 100%. Mp 162 °C.

*(2s,4s)-2-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**2d**).* A mixture of **24** (43.0 mg, 0.25 mmol), **35** (70.4 mg, 0.25 mmol), HOBt-H_2O (46.2 mg, 0.30 mmol), EDCI (57.8 mg, 0.30 mmol), DIPEA (0.158 mL, 0.90 mmol), and DMF (1.5 mL) was stirred at rt for 18 h. After addition of water, the resultant precipitate was collected, washed with water, and dried in vacuo. The crude solid was recrystallized from EtOH (8 mL)/H_2O (5 mL) to give **2d** (69 mg, 0.174 mmol, 69%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04−1.27 (2H, m), 1.70−1.87 (2H, m), 1.93−2.11 (1H, m), 2.23−2.66 (5H, m), 2.90−3.08 (2H, m), 3.76−3.87 (1H, m), 3.91 (2H, d, J = 6.2 Hz), 4.32−4.42 (3H, m), 7.12−7.20 (2H, m), 7.38−7.46 (1H, m), 8.02 (1H, s). LCMS (ESI/APCI) m/z 397.0 $[M + H]^+$. Anal. Calcd for $C_{19}H_{22}ClFN_2O_4$ H_2O: C, 55.01; H, 5.83; N, 6.75. Found: C, 55.31; H, 5.82; N, 6.76. HPLC purity 99.9%. Mp 170 °C.

*7-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-1,7-diazaspiro[4.4]nonan-2-one (**2e**).* To a cooled (−10 °C)

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

mixture of **35** (140 mg, 0.5 mmol) and MeCN (4 mL) were added DIPEA (0.523 mL, 3.00 mmol) and triphosgene (51.9 mg, 0.18 mmol). The mixture was stirred at the same temperature for 30 min. To the mixture was added 1,7-diazaspiro[4.4]nonan-2-one hydrochloride (88 mg, 0.50 mmol), and the mixture was stirred at rt for 2 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 50−100% EtOAc in hexane to 20% MeOH in EtOAc). The residue was triturated with EtOAc-hexane to give **2e** (63.0 mg, 0.154 mmol, 31%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (2H, d, J = 5.7 Hz), 1.66−1.87 (4H, m), 1.87−2.03 (3H, m), 2.13−2.38 (2H, m), 2.71 (2H, t, J = 12.6 Hz), 3.12−3.29 (2H, m), 3.39 (2H, t, J = 6.7 Hz), 3.69 (2H, d, J = 12.1 Hz), 3.91 (2H, d, J = 6.4 Hz), 7.17 (2H, d, J = 5.4 Hz), 7.42 (1H, d, J = 8.3 Hz), 8.01 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.7, 28.8, 30.5, 31.3, 36.1, 37.3, 45.8, 46.8, 58.5, 63.7, 73.9, 114.9, 115.17, 115.20, 115.3, 117.4, 117.7, 122.4, 122.5, 151.25, 151.27, 154.9, 157.3, 162.1, 176.2. LCMS (ESI/APCI) m/z 410.1 [M + H]$^+$. Anal. Calcd for $C_{20}H_{25}ClFN_3O_3$: C, 58.61; H, 6.15; N, 10.25. Found: C, 58.43; H, 6.24; N, 10.18. HPLC purity 99.9%. Mp 150 °C.

*7-(4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carbonyl)-1,7-diazaspiro[4.5]decan-2-one (**2f**).* To a stirred suspension of **35** (140 mg, 0.50 mmol) in THF (5 mL) was added 4-nitrophenyl chloroformate (111 mg, 0.55 mmol) at 0 °C, and then Et_3N (0.278 mL, 2.00 mmol) was added dropwise thereto at 0 °C. The mixture was allowed to warm up to rt and stirred for 1h. To this mixture were added **32** (249 mg, 0.5 mmol) and Et_3N (0.278 mL, 2.00 mmol) in THF (5 mL), and the mixture was heated to reflux overnight. The mixture was diluted with water and extracted with EtOAc. The extract was washed with sat.$NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 50−100% EtOAc in hexane to 30% MeOH in EtOAc). The residue was triturated with EtOAc-hexane to give **2f** (10.0 mg, 0.024 mmol, 5%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20−1.37 (2H, m), 1.45−1.61 (3H, m), 1.61−2.02 (6H, m), 2.06−2.30 (2H, m), 2.66−2.82 (2H, m), 2.84−3.05 (3H, m), 3.06−3.18 (1H, m), 3.54−3.71 (2H, m), 3.92 (2H, d, J = 6.3 Hz), 7.16 (2H, dd, J = 6.7, 1.7 Hz), 7.42 (1H, dt, J = 8.2, 1.7 Hz), 7.92 (1H, s). LCMS (ESI/APCI) m/z 424.1 [M + H]$^+$. HPLC purity 99.1%.

*(2s,4s)-2-(4-((2-Chloro-4-fluorophenoxy)methyl)-4-methylpiperidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3a**).* To a stirred mixture of **24** (46.5 mg, 0.27 mmol), **38** (80 mg, 0.27 mmol), EDCI (62.6 mg, 0.33 mmol), HOBt-H_2O (41.6 mg, 0.27 mmol), and MeCN (2 mL) was added Et_3N (0.076 mL, 0.54 mmol) at rt, and the mixture was stirred for 3h. The mixture was diluted with aq. $NaHCO_3$, and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with MeOH/EtOAc (0−5%), and crystallized from EtOAc/hexane to give **3a** (80 mg, 0.195 mmol, 72%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (3H, s), 1.42−1.57 (2H, m), 1.67 (2H, ddd, J = 13.8, 9.4, 4.5 Hz), 2.40−2.57 (2H, m), 2.58−2.80 (2H, m), 3.02 (1H, quin, J = 7.9 Hz), 3.29 (2H, dtd, J = 13.5, 9.8, 3.5 Hz), 3.44−3.61 (1H, m), 3.70 (2H, d, J = 0.9 Hz), 4.07 (1H, dt, J = 13.7, 4.7 Hz), 4.37 (2H, s), 5.80 (1H, s), 6.77−6.87 (1H, m), 6.88−7.00 (1H, m), 7.13 (1H, dd, J = 8.0, 2.9 Hz). LCMS (ESI/APCI) m/z 411.2 [M + H]$^+$. HPLC purity 99.0%.

*(2s,4s)-2-(4-((R or S)-1-(2-Chloro-4-fluorophenoxy)ethyl)-piperidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3b**).* To a mixture of **23b** (60 mg, 0.30 mmol), THF (1 mL), and MeOH (0.5 mL) was added 2 M NaOH (0.181 mL, 0.36 mmol), and the mixture was stirred at rt for 1 h. The mixture was neutralized with 2 M HCl (0.181 mL), and the mixture was concentrated in vacuo. The residue was diluted with DMF (2 mL), followed by the addition of **41a** (129 mg, 0.30 mmol), HOBt-H_2O (55.4 mg, 0.36 mmol), EDCI (69.3 mg, 0.36 mmol) and DIPEA (0.189 mL, 1.08 mmol) at rt. The mixture was stirred at rt for 4 h. The mixture was poured into sat.$NaHCO_3$, and extracted with EtOAc. The organic layer was separated, washed with and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−20% MeOH in EtOAc). The residue was triturated with EtOAc-hexane to give ***rac*-3b** (100 mg). This was optically resolved by preparative HPLC (CHIRALPAK ID, 50 mm I.D. × 500 mm L., 5 μm particle size, Daicel, Japan, eluent:hexane/IPA = 150:850 v/v, flow rate: 60 mL/min, temp.: 30 °C). The fractions of tR1 were concentrated, and the residual solid was recrystallized from IPA/EtOAc/hexane to give **3b** (31 mg, 0.075 mmol, >99.9% ee, 3 steps 25% from **23b**) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03−1.30 (5H, m), 1.67 (1H, d, J = 12.7 Hz), 1.82 (2H, d, J = 7.6 Hz), 2.19−2.43 (5H, m), 2.80−3.06 (2H, m), 3.83 (1H, d, J = 13.0 Hz), 4.21−4.57 (4H, m), 7.01−7.28 (2H, m), 7.40 (1H, dd, J = 8.3, 2.6 Hz), 8.02 (1H, s). LCMS (ESI/APCI) m/z 411.2 [M + H]$^+$. Anal. Calcd for $C_{20}H_{24}ClFN_2O_4$: C, 58.47; H, 5.89; N, 6.82. Found: C, 58.07; H, 5.77; N, 6.80. HPLC purity 100%.

*(2s,4s)-2-(4-((R or S)-1-(2-Chloro-4-fluorophenoxy)propyl)-piperidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3c**).* A mixture of **41b** (127 mg, 0.47 mmol), **24** (80 mg, 0.47 mmol), EDCI (134 mg, 0.70 mmol), HOBt (76 mg, 0.56 mmol), Et_3N (0.326 mL, 2.34 mmol), and DMF (5 mL) was stirred at rt overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 0−10% MeOH in EtOAc) to give ***rac*-3c** (145 mg). This was optically resolved by preparative SFC (CHIRALPAK IA, 20 mm I.D. × 250 mm L., 5 μm particle size, Daicel, Japan, eluent: CO_2/EtOH = 700:300 v/v, flow rate: 50 mL/min, temp.: 35 °C). The fractions of tR1 were concentrated to give **3c** (47 mg, 0.111 mmol, >99.9% ee, 2 steps 24%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.3 Hz), 1.03−1.33 (2H, m), 1.50−1.97 (5H, m), 2.21−2.59 (5H, m), 2.82−3.10 (2H, m), 3.81 (1H, d, J = 13.2 Hz), 4.23 (1H, d, J = 4.9 Hz), 4.36 (3H, s), 7.08−7.27 (2H, m), 7.40 (1H, dd, J = 8.3, 3.0 Hz), 8.04 (1H, s). LCMS (ESI/APCI) m/z 425.2 [M + H]$^+$. HPLC purity 100%.

*(2s,4s)-2-(4-((R or S)-1-(2-Chloro-4-fluorophenoxy)-2,2,2-trifluoroethyl)piperidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3d**).* A mixture of **41c** (146 mg, 0.47 mmol), **24** (80 mg, 0.47 mmol), EDCI (134 mg, 0.70 mmol), HOBt (76 mg, 0.56 mmol), Et_3N (0.326 mL, 2.34 mmol), and DMF (5 mL) was stirred at rt overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 0−10% MeOH in EtOAc) to give ***rac*-3d** (133 mg). This was optically resolved by preparative HPLC (CHIRALPAK AD, 50 mm I.D. × 500 mm L., 5 μm particle size, Daicel, Japan, eluent:hexane/IPA = 800/200 v/v, flow rate: 80 mL/min, temp.: 30 °C). The fractions of tR1 were concentrated to give **3d** (38 mg, 0.082 mmol, >99.9% ee, 2 steps 17%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.53 (2H, m), 1.68−1.86 (2H, m), 2.13−2.64 (6H, m), 2.90−3.09 (2H, m), 3.81 (1H, brs), 4.27−4.48 (3H, m), 5.18 (1H, t, J = 5.1 Hz), 7.24 (1H, ddd, J = 9.3, 8.1, 3.0 Hz), 7.40 (1H, dd, J = 9.3, 4.9 Hz), 7.49 (1H, dd, J = 8.3, 3.0 Hz), 8.03 (1H, d, J = 1.5 Hz). LCMS (ESI/APCI) m/z 465.1 [M + H]$^+$. HPLC purity 96.7%.

*(2s,4s)-2-(4-((2-Chloro-4-fluorobenzyl)oxy)piperidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3e**).* EDCI (240 mg, 1.25 mmol) was added to a solution of **44a** (400 mg, 0.96 mmol), **24** (181 mg, 1.06 mmol), HOBt (169 mg, 1.25 mmol), and Et_3N (0.201 mL, 1.44 mmol) in DMF (2.5 mL) at 0 °C, and the reaction mixture was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with 1 M HCl, 10% K_2CO_3, and brine, and dried over Na_2SO_4. The solution was passed through NH silica gel and concentrated in vacuo. The residue was solidified with hexane−EtOAc (3:1 v/v) to give **3e** (338 mg, 0.852 mmol,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

89%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32−1.50 (2H, m), 1.76−1.92 (2H, m), 2.24−2.45 (4H, m), 2.94−3.23 (3H, m), 3.52−3.73 (2H, m), 3.76−3.88 (1H, m), 4.36 (2H, s), 4.56 (2H, s), 7.18−7.27 (1H, m), 7.44 (1H, dd, J = 8.9, 2.6 Hz), 7.56 (1H, dd, J = 8.5, 6.4 Hz), 8.02 (1H, s). LCMS (ESI/APCI) m/z 397.1 [M + H]$^+$. HPLC purity 100%.

*(2s,4s)-2-(3-((2-Chloro-4-fluorobenzyl)oxy)pyrrolidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3f**).* EDCI (496 mg, 2.59 mmol) was added to a solution of **44b** (800 mg, 1.99 mmol), **24** (375 mg, 2.19 mmol), HOBt (350 mg, 2.59 mmol), and Et_3N (0.416 mL, 2.99 mmol) in DMF (5 mL) at 0 °C, and the reaction mixture was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with 1 M HCl, 10% K_2CO_3, and brine, and dried over Na_2SO_4. The solution was passed through NH silica gel and concentrated in vacuo. The residue was solidified with hexane−EtOAc (3:1 v/v) to give ***rac*-3f** (594 mg, 1.55 mmol, 78%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.84−2.17 (2H, m), 2.24−2.44 (4H, m), 2.82−2.99 (1H, m), 3.23−3.55 (4H, m), 4.14−4.29 (1H, m), 4.36 (2H, s), 4.46−4.58 (2H, m), 7.18−7.28 (1H, m), 7.41−7.48 (1H, m), 7.48−7.56 (1H, m), 8.06 (1H, s). LCMS (ESI/APCI) m/z 383.1 [M + H]$^+$. HPLC purity 99.1%.

*(2s,4s)-2-(3-((2-Chloro-4-fluorophenoxy)methyl)pyrrolidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3g**).* EDCI (310 mg, 1.62 mmol) was added to a solution of **41d** (500 mg, 1.24 mmol), **24** (234 mg, 1.37 mmol), HOBt (219 mg, 1.62 mmol), and Et_3N (0.260 mL, 1.87 mmol) in DMF (4 mL) at 0 °C, and the reaction mixture was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with 1 M HCl, 10% K_2CO_3, and brine, and dried over Na_2SO_4. The solution was passed through NH silica gel and concentrated in vacuo. The residue was solidified with hexane−EtOAc (3:1 v/v) to give ***rac*-3g** (407 mg, 1.06 mmol, 85%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64−1.88 (1H, m), 1.93−2.15 (1H, m), 2.24−2.45 (4H, m), 2.56−2.77 (1H, m), 2.82−2.98 (1H, m), 3.13−3.63 (4H, m), 3.96−4.09 (2H, m), 4.36 (2H, s), 7.14−7.21 (2H, m), 7.39−7.46 (1H, m), 8.06 (1H, s). LCMS (ESI/APCI) m/z 383.1 [M + H]$^+$. HPLC purity 100%.

*(2s,4s)-2-(3-((2-Chloro-4-fluorophenoxy)methyl)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3h**).* EDCI (163 mg, 0.85 mmol) was added to a mixture of **41e** (300 mg, 0.77 mmol), **24** (132 mg, 0.77 mmol), DIPEA (0.446 mL, 2.55 mmol) and HOBt-H_2O (130 mg, 0.85 mmol) in DMF (6 mL) at rt. The mixture was stirred at rt overnight. EtOAc and water were added to the reaction mixture at rt, then the organic layer was separated. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was collected, washed with EtOAc and dried to give **3h** (90 mg, 0.244 mmol, 32%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21−2.41 (4H, m), 2.66−2.82 (1H, m), 3.02 (1H, tt, J = 8.4, 5.7 Hz), 3.71 (1H, dd, J = 9.5, 5.6 Hz), 3.88−3.99 (2H, m), 4.14−4.22 (3H, m), 4.35 (2H, s), 7.15−7.25 (2H, m), 7.39−7.47 (1H, m), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.1, 28.2, 38.07, 38.12, 50.2, 52.2, 54.9, 71.0, 75.7, 115.0, 115.2, 115.75, 115.84, 117.4, 117.7, 122.6, 122.7, 151.08, 151.11, 155.2, 157.6, 158.0, 173.1. LCMS (ESI/APCI) m/z 369.1 [M + H]$^+$. Anal. Calcd for $C_{17}H_{18}ClFN_2O_4$: C, 55.37; H, 4.92; N, 7.60. Found: C, 55.52; H, 5.12; N, 7.62. HPLC purity 100%. Mp 190 °C.

*(2s,4s)-2-(3-((2-Chloro-4-fluorobenzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**3i**).* EDCI (257 mg, 1.34 mmol) was added to a solution of **44c** (400 mg, 1.03 mmol), **24** (194 mg, 1.13 mmol), HOBt (181 mg, 1.34 mmol), and Et_3N (0.216 mL, 1.55 mmol) in DMF (2.5 mL) at 0 °C, and the reaction mixture was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with 1 M HCl, 10% K_2CO_3, and brine, and dried over Na_2SO_4. The solution was passed through NH silica gel and concentrated in vacuo. The residue was solidified with hexane−EtOAc (3:1 v/v) to give **3i** (270 mg, 0.732 mmol, 71%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18−2.41 (4H, m), 2.64−2.81 (1H, m), 3.61−3.73 (1H, m), 3.86−3.97 (1H, m), 3.97−4.09 (1H, m), 4.19−4.30 (1H, m), 4.34 (2H, s), 4.37−4.47 (1H, m), 4.50 (2H, s), 7.18−7.31 (1H, m), 7.47 (1H, dd, J = 8.9, 2.5 Hz), 7.57 (1H, dd, J = 8.3, 6.6 Hz), 8.08 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.4, 38.1, 54.9, 55.3, 57.0, 67.2, 67.8, 75.7, 114.7, 114.9, 116.9, 117.2, 131.90, 131.93, 132.2, 132.3, 133.9, 134.0, 158.0, 160.8, 163.3, 173.2. LCMS (ESI/APCI) m/z 369.1 [M + H]$^+$. Anal. Calcd for $C_{17}H_{18}ClFN_2O_4$: C, 55.37; H, 4.92; N, 7.60. Found: C, 55.45; H, 5.05; N, 7.63. HPLC purity 100%. Mp 128 °C.

*(2s,4s)-2-(3-((2-Chloro-4-methylbenzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4a**).* A mixture of **46a** (500 mg, 1.30 mmol), **24** (223 mg, 1.30 mmol), EDCI (275 mg, 1.43 mmol), HOBt-H_2O (219 mg, 1.43 mmol), DIPEA (0.682 mL, 3.91 mmol), and DMF (4.34 mL) was stirred at rt for 3 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE/EtOAc to give **4a** (279 mg, 0.765 mmol, 59%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18−2.40 (7H, m), 2.64−2.80 (1H, m), 3.65 (1H, dd, J = 10.5, 3.9 Hz), 3.91 (1H, dd, J = 9.3, 3.2 Hz), 4.01 (1H, dd, J = 10.3, 6.5 Hz), 4.19−4.29 (1H, m), 4.34 (2H, s), 4.36−4.45 (1H, m), 4.47 (2H, s), 7.17 (1H, d, J = 7.6 Hz), 7.30 (1H, s), 7.39 (1H, d, J = 7.7 Hz), 8.08 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.8, 26.3, 38.1, 54.9, 55.4, 57.1, 67.6, 67.7, 75.7, 128.4, 130.0, 130.6, 132.3, 132.8, 140.1, 158.0, 173.2. LCMS (ESI/APCI) m/z 365.1 [M + H]$^+$. Anal. Calcd for $C_{18}H_{21}ClN_2O_4$: C, 59.26; H, 5.80; N, 7.68. Found: C, 59.07; H, 5.77; N, 7.69. HPLC purity 99.6%. Mp 129 °C.

*(2s,4s)-2-(3-((2,4-Dichlorobenzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4b**).* A mixture of **48a** (564 mg, 1.39 mmol), **24** (239 mg, 1.39 mmol), EDCI (294 mg, 1.53 mmol), HOBt-H_2O (235 mg, 1.53 mmol), DIPEA (0.731 mL, 4.18 mmol), and DMF (4.65 mL) was stirred at rt for 16 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE to give **4b** (261 mg, 0.677 mmol, 49%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.16−2.40 (4H, m), 2.71 (1H, q, J = 9.1 Hz), 3.68 (1H, dd, J = 10.1, 3.5 Hz), 3.93 (1H, dd, J = 9.6, 4.0 Hz), 4.03 (1H, dd, J = 10.5, 6.5 Hz), 4.26 (1H, dd, J = 8.6, 7.1 Hz), 4.34 (2H, s), 4.38−4.47 (1H, m), 4.50 (2H, s), 7.43−7.50 (1H, m), 7.50−7.58 (1H, m), 7.64 (1H, d, J = 2.1 Hz), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.4, 38.1, 54.9, 55.3, 57.0, 67.1, 67.9, 75.7, 127.9, 129.2, 131.7, 133.7, 133.8, 134.7, 158.0, 173.2. LCMS (ESI/APCI) m/z 385.1 [M + H]$^+$. Anal. Calcd for $C_{17}H_{18}Cl_2N_2O_4$: C, 53.00; H, 4.71; N, 7.27. Found: C, 53.00; H, 4.68; N, 7.31. HPLC purity 100%. Mp 128 °C.

*(2s,4s)-2-(3-((2-Chloro-4-(trifluoromethyl)benzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4c**).* To a stirred mixture of **24** (78 mg, 0.46 mmol), **48b** (200 mg, 0.46 mmol), EDCI (105 mg, 0.55 mmol), HOBt-H_2O (69.9 mg, 0.46 mmol), and MeCN (3 mL) was added dropwise Et_3N (0.152 mL, 1.10 mmol) at rt, and the mixture was stirred at rt overnight. The mixture was diluted with EtOAc, washed with aq. $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with MeOH/EtOAc (0−9%) to give crude amorphous powder (152.0 mg). This material was crystallized from EtOAc/hexane to give **4c** (136 mg, 0.325 mmol, 71%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39−2.52 (2H, m), 2.53−2.65 (2H, m), 2.65−2.81 (1H, m), 3.95−4.13 (2H, m), 4.20−4.38 (4H, m), 4.47 (1H, tt, J = 6.4, 4.1 Hz), 4.60 (2H, s), 6.22 (1H, brs), 7.48−7.81 (3H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.4, 38.1, 54.9, 55.3, 57.0, 67.1, 68.2, 75.7, 123.8, 124.6, 126.4, 130.3, 130.6, 133.2, 140.5, 158.0, 173.2. LCMS (ESI/APCI) m/z 419.1 [M + H]$^+$. Anal. Calcd for $C_{18}H_{18}ClF_3N_2O_4$: C, 51.62; H, 4.33; N, 6.69. Found: C, 51.49; H, 4.42; N, 6.79. HPLC purity 99.6%. Mp 114 °C.

*(2s,4s)-2-(3-((3-Chloro-4-(trifluoromethyl)benzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4d**).* A mixture of **46b** (351 mg, 0.80 mmol), **24** (137 mg, 0.80 mmol), EDCI (169 mg, 0.88 mmol), HOBt-H_2O (135 mg, 0.88 mmol), DIPEA (0.420 mL, 2.40 mmol), and DMF (2.67 mL) was stirred at rt for 3 h. The

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE/EtOAc to give **4d** (149 mg, 0.356 mmol, 44%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20−2.39 (4H, m), 2.69−2.79 (1H, m), 3.71 (1H, dd, J = 10.5, 3.7 Hz), 3.95 (1H, dd, J = 9.7, 3.7 Hz), 3.99−4.08 (1H, m), 4.21−4.30 (1H, m), 4.34 (2H, s), 4.38−4.46 (1H, m), 4.55 (2H, s), 7.52 (1H, d, J = 8.7 Hz), 7.70 (1H, s), 7.86 (1H, d, J = 8.3 Hz), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.4, 38.1, 54.9, 55.3, 57.0, 67.9, 68.6, 75.7, 123.4, 126.1, 126.9, 128.4, 130.6, 131.18, 131.19, 145.3, 158.0, 173.2. LCMS (ESI/APCI) m/z 419.2 $[M + H]^+$. Anal. Calcd for $C_{18}H_{18}ClF_3N_2O_4$: C, 51.62; H, 4.33; N, 6.69. Found: C, 51.46; H, 4.39; N, 6.65. HPLC purity 100%. Mp 126 °C.

*(2s,4s)-2-(3-((3,4-Dichlorobenzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4e**).* A mixture of **48c** (547 mg, 1.35 mmol), **24** (232 mg, 1.35 mmol), EDCI (285 mg, 1.49 mmol), HOBt-H_2O (228 mg, 1.49 mmol), DIPEA (0.709 mL, 4.06 mmol), and DMF (4.51 mL) was stirred at rt for 16 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE to give **4e** (248 mg, 0.644 mmol, 48%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18−2.40 (4H, m), 2.72 (1H, t, J = 8.5 Hz), 3.68 (1H, dd, J = 10.3, 4.1 Hz), 3.92 (1H, dd, J = 9.3, 3.9 Hz), 4.01 (1H, dd, J = 9.7, 7.1 Hz), 4.19−4.28 (1H, m), 4.34 (2H, s), 4.35−4.42 (1H, m), 4.44 (2H, s), 7.35 (1H, dd, J = 8.3, 1.9 Hz), 7.59−7.66 (2H, m), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.3, 38.1, 54.9, 55.4, 57.1, 67.7, 68.8, 75.7, 128.5, 130.1, 130.7, 131.0, 131.5, 139.4, 158.0, 173.1. LCMS (ESI/APCI) m/z 385.1 $[M + H]^+$. Anal. Calcd for $C_{17}H_{18}Cl_2N_2O_4$: C, 53.00; H, 4.71; N, 7.27. Found: C, 52.93; H, 4.69; N, 7.28. HPLC purity 100%. Mp 154 °C.

*(2s,4s)-2-(3-((3-Chloro-4-methylbenzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4f**).* A mixture of **46e** (14.0 g, 36.5 mmol), **24** (6.24 g, 36.5 mmol), EDCI (7.69 g, 40.1 mmol), HOBt-H_2O (6.14 g, 40.1 mmol), DIPEA (19.1 mL, 109 mmol), and DMF (91 mL) was stirred at rt for 16 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo to give a crude material (10.0 g). This material was dissolved in hot EtOH (40 mL) and hexane/i-Pr_2O (1:1 v/v, 80 mL) was added to the mixture. The obtained white crystal was collected by filtration and dried in vacuo to give **4f** (8.72 g, 23.9 mmol, 66%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18−2.40 (7H, m), 2.72 (1H, quin, J = 8.9 Hz), 3.65 (1H, dd, J = 10.1, 3.9 Hz), 3.90 (1H, dd, J = 9.4, 3.4 Hz), 4.00 (1H, dd, J = 10.5, 6.5 Hz), 4.18−4.28 (1H, m), 4.30−4.44 (5H, m), 7.21 (1H, dd, J = 7.7, 1.5 Hz), 7.33 (1H, d, J = 7.7 Hz), 7.39 (1H, d, J = 1.5 Hz), 8.08 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.7, 26.3, 38.1, 54.9, 55.4, 57.1, 67.5, 69.4, 75.7, 127.1, 128.6, 131.6, 133.6, 135.2, 137.9, 158.0, 173.1. LCMS (ESI/APCI) m/z 365.1 $[M + H]^+$. Anal. Calcd for $C_{18}H_{21}ClN_2O_4$: C, 59.26; H, 5.80; N, 7.68. Found: C, 59.18; H, 5.93; N, 7.54. HPLC purity 99.7%. Mp 118 °C.

*(2s,4s)-2-(3-((3,4-Bis(trifluoromethyl)benzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4g**).* A mixture of **50** (500 mg, 1.06 mmol), **24** (182 mg, 1.06 mmol), EDCI (224 mg, 1.17 mmol), HOBt-H_2O (179 mg, 1.17 mmol), DIPEA (0.556 mL, 3.18 mmol), and DMF (3.54 mL) was stirred at rt for 16 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE/EtOAc to give **4g** (239 mg, 0.528 mmol, 50%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20−2.40 (4H, m), 2.66−2.81 (1H, m), 3.73 (1H, dd, J = 10.6, 4.0 Hz), 3.92−4.10 (2H, m), 4.28 (1H, dd, J = 8.2, 6.5 Hz), 4.34 (2H, s), 4.39−4.50 (1H, m), 4.64 (2H, s), 7.89 (1H, d, J = 8.1 Hz), 7.99 (1H, s), 8.02-8.14 (2H, m). LCMS (ESI/APCI) m/z 453.1 $[M + H]^+$. Anal. Calcd for $C_{19}H_{18}F_6N_2O_4$: C, 50.45; H, 4.01; N, 6.19. Found: C, 50.47; H, 4.27; N, 6.29. HPLC purity 99.3%. Mp 141 °C.

*(2s,4s)-2-(3-((4-Chloro-3-(trifluoromethyl)benzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4h**).* To a stirred mixture of **24** (78 mg, 0.46 mmol), **46f** (200 mg, 0.46 mmol), EDCI (114 mg, 0.59 mmol), HOBt-H_2O (69.9 mg, 0.46 mmol), and MeCN (4 mL) was added dropwise Et_3N (0.152 mL, 1.10 mmol) at rt, and the mixture was stirred at rt overnight. The mixture was diluted with EtOAc, washed with aq. $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with MeOH/EtOAc (0−9%) and crystallized from EtOAc/IPE to give **4h** (148 mg, 0.354 mmol, 78%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20−2.41 (4H, m), 2.64−2.83 (1H, m), 3.69 (1H, dd, J = 10.4, 4.0 Hz), 3.94 (1H, dd, J = 9.0, 3.8 Hz), 4.02 (1H, dd, J = 10.4, 6.6 Hz), 4.21−4.31 (1H, m), 4.34 (2H, s), 4.37−4.48 (1H, m), 4.52 (2H, s), 7.62−7.78 (2H, m), 7.83 (1H, d, J = 1.5 Hz), 8.10 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.4, 38.1, 54.9, 55.3, 57.0, 67.8, 68.7, 75.7, 119.2, 121.9, 124.7, 126.5, 126.8, 127.2, 127.4, 130.28, 130.30, 132.1, 133.8, 138.4, 158.0, 173.2. LCMS (ESI/APCI) m/z 419.1 $[M + H]^+$. Anal. Calcd for $C_{18}H_{18}ClF_3N_2O_4$: C, 51.62; H, 4.33; N, 6.69. Found: C, 51.48; H, 4.58; N, 6.74. HPLC purity 100%. Mp 149 °C.

*(2s,4s)-2-(3-((4-Methyl-3-(trifluoromethyl)benzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4i**).* A mixture of **46c** (500 mg, 1.20 mmol), **24** (205 mg, 1.20 mmol), EDCI (253 mg, 1.32 mmol), HOBt-H_2O (202 mg, 1.32 mmol), DIPEA (0.628 mL, 3.59 mmol), and DMF (3.99 mL) was stirred at rt for 64 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE/EtOAc to give **4i** (302 mg, 0.758 mmol, 63%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.19−2.39 (4H, m), 2.44 (3H, d, J = 1.9 Hz), 2.64−2.80 (1H, m), 3.66 (1H, dd, J = 10.4, 4.0 Hz), 3.92 (1H, dd, J = 9.2, 3.6 Hz), 4.01 (1H, dd, J = 10.7, 6.2 Hz), 4.20−4.29 (1H, m), 4.31−4.43 (3H, m), 4.48 (2H, s), 7.40−7.47 (1H, m), 7.50−7.57 (1H, m), 7.63 (1H, s), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 18.92, 18.94, 26.3, 38.1, 54.9, 55.4, 57.1, 67.6, 69.4, 75.7, 125.0, 125.4, 127.8, 132.3, 132.7, 135.89, 135.90, 136.5, 158.0, 173.2. LCMS (ESI/APCI) m/z 399.2 $[M + H]^+$. Anal. Calcd for $C_{19}H_{21}F_3N_2O_4$: C, 57.28; H, 5.31; N, 7.03. Found: C, 56.89; H, 5.61; N, 7.01. HPLC purity 100%. Mp 133 °C.

*(2s,4s)-2-(3-((3-Methyl-4-(trifluoromethyl)benzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4j**).* A mixture of **46g** (268 mg, 0.64 mmol), **24** (110 mg, 0.64 mmol), EDCI (135 mg, 0.71 mmol), HOBt-H_2O (108 mg, 0.71 mmol), DIPEA (0.336 mL, 1.93 mmol), and DMF (2.14 mL) was stirred at rt for 3 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE/EtOAc to give **4j** (149 mg, 0.374 mmol, 58%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18−2.39 (4H, m), 2.44 (3H, d, J = 1.7 Hz), 2.68−2.78 (1H, m), 3.69 (1H, dd, J = 10.5, 3.7 Hz), 3.93 (1H, dd, J = 8.8, 4.1 Hz), 3.98−4.08 (1H, m), 4.20−4.30 (1H, m), 4.34 (2H, s), 4.36−4.44 (1H, m), 4.49 (2H, s), 7.35 (1H, d, J = 7.6 Hz), 7.40 (1H, s), 7.65 (1H, d, J = 8.1 Hz), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.16, 19.17, 26.4, 38.1, 54.9, 55.4, 57.1, 67.8, 69.4, 75.7, 125.1, 125.7, 126.2, 127.1, 131.6, 136.60, 136.61, 142.7, 158.0, 173.2. LCMS (ESI/APCI) m/z 399.2 $[M + H]^+$. Anal. Calcd for $C_{19}H_{21}F_3N_2O_4$: C, 57.28; H, 5.31; N, 7.03. Found: C, 57.04; H, 5.42; N, 7.01. HPLC purity 99.2%. Mp 138 °C.

*(2s,4s)-2-(3-((4-Chloro-3-methylbenzyl)oxy)azetidine-1-carbonyl)-7-oxa-5-azaspiro[3.4]octan-6-one (**4k**).* A mixture of **46d** (500 mg, 1.30 mmol), **24** (223 mg, 1.30 mmol), EDCI (275 mg, 1.43 mmol), HOBt-H_2O (219 mg, 1.43 mmol), DIPEA (0.682 mL, 3.91 mmol), and DMF (4.34 mL) was stirred at rt for 3 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, passed through a short pad of

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

NH silica gel, and concentrated in vacuo. The resultant residue was washed with IPE to give **4k** (152 mg, 0.417 mmol, 32%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20−2.37 (7H, m), 2.67−2.77 (1H, m), 3.65 (1H, dd, J = 10.2, 3.8 Hz), 3.90 (1H, dd, J = 9.6, 4.2 Hz), 4.00 (1H, dd, J = 10.3, 6.3 Hz), 4.19−4.27 (1H, m), 4.28−4.43 (5H, m), 7.18 (1H, dd, J = 8.3, 1.9 Hz), 7.32 (1H, s), 7.39 (1H, d, J = 8.1 Hz), 8.09 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.0, 26.3, 38.1, 54.9, 55.4, 57.1, 67.5, 69.6, 75.7, 127.6, 129.2, 131.2, 133.0, 135.8, 137.1, 158.0, 173.1. LCMS (ESI/APCI) m/z 365.2 [M + H]$^+$. Anal. Calcd for $C_{18}H_{21}ClN_2O_4$: C, 59.26; H, 5.80; N, 7.68. Found: C, 59.43; H, 5.94; N, 7.72. HPLC purity 100%. Mp 146 °C.

*2-Oxo-2,3-dihydro-1H-pyrido[3,4-b][1,4]oxazine-7-carboxylic Acid (**6**).* To a solution of **5** (0.15 g, 0.83 mmol) in t-BuOH/H_2O (10/10 mL) were added 2-methyl-2-butene (0.5 mL), $NaClO_2$ (0.45 g, 5.0 mmol) and NaH_2PO_4 (0.21 g, 1.3 mmol), the mixture was stirred at rt for 3 h. After being concentrated in vacuo, the mixture was purified by Pre-HPLC [Gilson-GX281; Column: Waters X-Bridge C18: 100 mm×30 mm 5 μm; Mobile Phase: from 70% [water + 10 mM TFA] and 30% [MeCN] to 50% [water + 10 mM TFA] and 50% [MeCN] in 8 min, then changed to 5% [water + 10 mM TFA] and 95% [MeCN] in 0.2 min and under this condition for 3.8 min; flow rate: 20 mL/min; Column temperature: rt] to give **6** (0.11 g, 0.57 mmol, 68%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.79 (2H, s), 7.57 (1H, s), 8.24 (1H, s), 11.26 (1H, s). LCMS (ESI/APCI) m/z 195.1 [M + H]$^+$.

*tert-Butyl 4-(((Benzyloxy)carbonyl)amino)-5-hydroxypentanoate (**8**).* To a stirred mixture of *N*-α-Carbobenzoxy-*DL*-glutamic acid γ-t-butyl ester **7** (1.00 g, 2.96 mmol) and Et_3N (0.618 mL, 4.45 mmol) in THF (10.0 mL) was added dropwise isobutyl chloroformate (0.461 mL, 3.56 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. To this mixture was added dropwise a solution of $NaBH_4$ (336 mg, 8.89 mmol) in water (3.00 mL), and the mixture was stirred at 0 °C for 30 min. The reaction was quenched with sat. NH_4Cl at 0 °C, and the mixture was stirred at rt for 30 min. The mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with EtOAc/hexane (10−80%) to give **8** (841 mg, 2.60 mmol, 88%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 1.42−1.56 (1H, m), 1.69−1.87 (1H, m), 2.11−2.26 (2H, m), 3.18−3.29 (1H, m), 3.33−3.38 (1H, m), 3.44 (1H, d, J = 5.3 Hz), 4.66 (1H, t, J = 5.7 Hz), 4.91−5.10 (2H, m), 6.97 (1H, d, J = 8.7 Hz), 7.25−7.44 (5H, m).

*tert-Butyl 4-Amino-5-hydroxypentanoate (**9**).* A mixture of **8** (840 mg, 2.60 mmol) and 10% Pd-C (50.0 mg, 0.47 mmol) in EtOAc (8.00 mL) was stirred at rt for 6 h under H_2 atmosphere. The mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc (8.00 mL), and 20% $Pd(OH)_2$-C (50.0 mg, 0.36 mmol) was added thereto under N_2 atmosphere. The mixture was stirred at rt under H_2 atmosphere for 6 h. The mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to give **9** (490 mg, 2.59 mmol, 100%) as a pale brown oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22−1.37 (1H, m), 1.39 (9H, s), 1.43−1.69 (3H, m), 2.12−2.38 (2H, m), 2.53−2.61 (1H, m), 3.06−3.27 (2H, m), 4.49 (1H, brs). LCMS (ESI/APCI) m/z 134.2 [M − t-Bu + H]$^+$.

*tert-Butyl 3-(5-Ooxomorpholin-3-yl)propanoate (**10**).* To a stirred solution of **9** (488 mg, 2.58 mmol) and Et_3N (0.430 mL, 3.09 mmol) in THF (8.00 mL) was added dropwise chloroacetyl chloride (0.205 mL, 2.58 mmol) at −78 °C, and the mixture was stirred at the same temperature for 30 min. To this mixture was added t-BuOK (817 mg, 6.19 mmol) at −78 °C, and the mixture was allowed to warm up to rt. After being stirred at the same temperature for 2 h, the mixture was diluted with aq.NH_4Cl and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with EtOAc/hexane (50−100%) and MeOH/EtOAc (5−9%) to give **10** (237 mg, 1.03 mmol, 40%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (9H, s), 1.54−1.79 (2H, m), 2.15−2.42 (2H, m), 3.36 (1H, dt, J = 6.5, 3.0 Hz), 3.42−3.53 (1H, m), 3.76 (1H, dd, J = 11.7, 3.8 Hz), 3.93 (2H, s), 8.11 (1H, brs). LCMS (ESI/APCI) m/z 174.1 [M − t-Bu + H]$^+$.

*3-(5-Oxomorpholin-3-yl)propanoic Acid (**11**).* Compound **10** (236 mg, 1.03 mmol) was dissolved in TFA (4.00 mL) at rt, and the mixture was stirred at rt overnight. The mixture was concentrated in vacuo, and the residue was azeotroped with toluene (2 times). The residue was crystallized from EtOAc/IPE/hexane to give **11** (165 mg, 0.953 mmol, 93%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56−1.82 (2H, m), 2.19−2.40 (2H, m), 3.35−3.42 (1H, m), 3.42−3.51 (1H, m), 3.77 (1H, dd, J = 11.3, 3.4 Hz), 3.93 (2H, s), 8.13 (1H, s), 12.11 (1H, s). LCMS (ESI/APCI) m/z 174.2 [M + HM + H]$^+$.

*tert-Butyl-3-(hydroxyimino)cyclobutanecarboxylate (**13a**).* To a stirred solution of **12a** (500 g, 2.94 mol) in EtOH (9 L) was added NaOAc (976 g, 11.8 mol) and hydroxylamine hydrochloride (409 g, 5.88 mol) at rt, and the reaction mixture was refluxed for 4 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give **13a** (500 g, 2.70 mmol, 92%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s), 3.09−3.16 (5H, m), 6.83 (1H, brs).

*Ethyl 3-(Hydroxyimino)cyclobutanecarboxylate (**13b**).* A mixture of **12b** (25.6 g, 180 mmol), hydroxylamine hydrochloride (15.0 g, 216 mmol), NaOAc (17.7 g, 216 mmol), and EtOH (200 mL) was refluxed for 18 h. After being cooled to rt, the precipitates were filtered, and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc and water, and then extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, filtered through a silica gel pad, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−60% EtOAc in hexane) to give **13b** (25.1 g, 160 mmol, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.2 Hz), 3.04−3.32 (5H, m), 4.19 (2H, q, J = 7.2 Hz), 7.73 (1H, s). LCMS (ESI/APCI) m/z 158.1 [M + H]$^+$.

*tert-Butyl 3-Nitrocyclobutanecarboxylate (**14**).* To a stirred suspension of UHP (305 g, 3.24 mol) in MeCN (2 L) was added dropwise a solution of TFAA (454 mL, 3.24 mol) in acetonitrile (1 L) at −10 °C over 1 h, and the reaction mixture was allowed to warm up to rt for 1 h. The reaction mixture was added to a solution of **13a** (200 g, 1.08 mol) and Na_2HPO_4 (1.08 kg, 7.57 mol) in MeCN (2 L) at 80 °C over 30 min. The reaction mixture was stirred at the same temperature for 30 min. After that, the reaction mixture was filtered through a Celite pad, and the filtrate was poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 6−10% EtOAc/petroleum ether) to afford **14** (140 g, 0.696 mol, cis/trans mixture, 64%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s), 2.71−2.91 (4H, m), 3.12−3.27 (1H, m), 4.78−4.82 (1H, m).

*Ethyl 3-Nitrocyclobutanecarboxylate (**15a** and **15b**).* To a mixture of **13b** (5.00 g, 31.8 mmol), Na_2HPO_4 (45.2 g, 318 mmol), UHP (8.98 g, 95.4 mmol), and MeCN (60 mL) was added TFAA (27.0 mL, 191 mmol) in MeCN (40 mL) dropwise over 20 min at 80 °C, and the mixture was stirred at the same temperature for 1.8 h. After being cooled to rt, the mixture was diluted with EtOAc and water, and then extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered through silica gel pad, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−40% EtOAc in hexane) to give **15a** (1.00 g, 5.77 mmol, 18%, minor single isomer, less polar on Si-TLC) and **15b** (1.38 g, 7.97 mmol, 25%, major single isomer, more polar on Si-TLC) as a pale yellow oil. **15a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3H, t, J = 7.2 Hz), 2.73−3.01 (4H, m), 3.21−3.34 (1H, m), 4.19 (2H, q, J = 7.2 Hz), 5.03−5.18 (1H, m); **15b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.2

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

Hz), 2.72−3.04 (5H, m), 4.19 (2H, q, J = 7.1 Hz), 4.77−4.92 (1H, m).

*(1r,3s)-tert-Butyl-3-(3-methoxy-3-oxopropyl)-3-nitrocyclobutanecarboxylate (**16**).* To a stirred solution of **14** (150 g, 0.750 mol) in MeCN (1.5 L) was added DBU (133 mL, 0.900 mol) at 0 °C, followed by the dropwise addition of methyl acrylate (77.3 mL, 0.900 mol) at 0 °C, and the reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 6−8% EtOAc/petroleum ether) to afford **16** (65 g, 0.226 mol, 30%, cis isomer) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (9H, s), 2.29−2.39 (4H, m), 2.49−2.53 (2H, m), 2.80 (1H, quin, J = 8.8 Hz), 2.92−2.97 (2H, m), 3.69 (3H, s). LCMS (ESI/APCI) m/z 230.1 $[M - t\text{-Bu} - H]^-$.

*(2r,4s)-tert-Butyl 6-Oxo-5-azaspiro[3.4]octane-2-carboxylate (**17**).* To a stirred solution of **16** (65.0 g, 0.230 mol) in MeOH (650 mL) was added $NiCl_2{\cdot}6H_2O$ (53.7 g, 0.230 mol) at rt, followed by portionwise addition of $NaBH_4$ (43.0 g, 1.13 mol) at −10 °C, and the reaction mixture was stirred at the same temperature for 1 h. After that, K_2CO_3 (125 g, 0.910 mol) in water was added thereto at below 10 °C and stirred at rt for 2 h. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was washed with n-pentane to afford **17** (40 g, 0.18 mmol, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s), 2.17−2.21 (2H, m), 2.33−2.39 (6H, m), 2.76 (1H, quin, J = 8.40 Hz), 5.98 (1H, brs). LCMS (ESI/APCI) m/z 226.2 $[M + H]^+$.

*(2r,4s)-6-Oxo-5-azaspiro[3.4]octane-2-carboxylic Acid (**18**).* Compound **17** (80.0 g, 0.36 mol) was added to TFA (800 mL) at 0 °C, and the reaction mixture was stirred at rt for 4 h. After that, the reaction mixture was concentrated in vacuo. The residue was azeotroped with toluene (three times) to give crude compound. The crude material was stirred with methanol, filtered, and dried in vacuo to afford **18** (52.0 g, 0.307 mol, 86%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.06−2.10 (2H, m), 2.12−2.15 (2H, m), 2.19−2.23 (2H, m), 2.27−2.31 (2H, m), 2.71−2.78 (1H, m), 7.97 (1H, s), 12.14 (1H, brs). LCMS (ESI/APCI) m/z 170.2 $[M + H]^+$.

*(1s,3r)- Ethyl 3-(3-Methoxy-3-oxopropyl)-3-nitrocyclobutane-1-carboxylate (**19**).* To a cooled (0 °C) solution of **15a** (518 mg, 2.99 mmol) and methyl acrylate (0.323 mL, 3.59 mmol) in MeCN (5 mL) was added DBU (0.224 mL, 1.50 mmol), and the mixture was stirred at the same temperature for 20 min. The reaction was quenched with aq. NH_4Cl and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−40% EtOAc in hexane) to give **19** (196 mg, 0.756 mmol, 25%) and its stereoisomer (533 mg, 2.056 mmol, 68%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.2 Hz), 2.24−2.33 (2H, m), 2.41−2.49 (2H, m), 2.55−2.66 (2H, m), 2.99−3.12 (2H, m), 3.15−3.29 (1H, m), 3.68 (3H, s), 4.18 (2H, q, J = 7.2 Hz).

*(2s,4r)-6-Oxo-5-azaspiro[3.4]octane-2-carboxylic acid (**21**).* To a cooled (−10 °C) mixture of **19** (195 mg, 0.75 mmol), $NiCl_2{\cdot}6H_2O$ (179 mg, 0.75 mmol), and MeOH (2 mL) was added $NaBH_4$ (142 mg, 3.76 mmol) portionwise (×3). After being stirred at the same temperature for 2 h, the reaction was quenched with aq. K_2CO_3 (416 mg in water (1 mL)) at 0 °C. The mixture was stirred at 0 °C for 3 h and at rt for 2 h, and then filtered through a Celite pad. The filtrate was concentrated in vacuo, and the residue was dissolved in THF (2 mL)/MeOH (2 mL)/water (1 mL). To the mixture was added 2 M NaOH (0.752 mL, 1.50 mmol), and the mixture was stirred at rt overnight. The mixture was acidified with 6 M HCl to pH 4 and concentrated in vacuo. The crude residue was subjected to the next reaction without further purification. LCMS (ESI/APCI) m/z 170.1$[M + H]^+$.

*(1s,3s)-tert-Butyl-3-(hydroxymethyl)-3-nitrocyclobutanecarboxylate (**22a**).* To a stirred solution of **14** (300 g, 1.49 mol) in MeCN (4 L) was added 37% (w/v) formaldehyde solution (243 mL, 2.99 mol) at rt, followed by the dropwise addition of Et_3N (211 mL, 1.57 mol) at 0 °C, and the reaction mixture was stirred at rt for 2 h. After concentration in vacuo, the crude compound was purified by column chromatography (silica gel, eluted with 15−35% EtOAc/petroleum ether) to afford **22a** (180 g, 0.778 mol, 52%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (9H, s), 2.57−2.62 (m, 3H), 2.79−2.86 (1H, m), 2.92−2.96 (2H, m), 4.02 (2H, d, J = 4.5 Hz). LCMS (ESI/APCI) m/z 174.1 $[M - t\text{-Bu} - H]^-$.

*(1s,3s)-Ethyl 3-(Hydroxymethyl)-3-nitrocyclobutane-1-carboxylate (**22b**).* To a mixture of **15b** (820 mg, 4.74 mmol), Et_3N (0.726 mL, 5.21 mmol), and MeCN (8 mL) was added 35% (w/v) formaldehyde solution (0.750 mL, 9.47 mmol), and the mixture was stirred at rt for 1 h. The mixture was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, eluted with 0−50% EtOAc in hexane) to give **22b** (452 mg, 2.22 mmol, 47%) and its stereoisomer (244 mg, 1.20 mmol, 25%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 2.34 (1H, brs), 2.58−2.71 (2H, m), 2.84−3.11 (3H, m), 4.05 (2H, brs), 4.18 (2H, q, J = 7.2 Hz). LCMS (ESI/APCI) m/z 174.1 $[M - t\text{-Bu} - H]^-$.

*(2s,4s)-tert-Butyl-6-oxo-7-oxa-5-azaspiro[3.4]octane-2-carboxylate (**23a**).* A mixture of **22a** (210 g, 0.908 mol) and Raney nickel (20.0 g) in isopropyl alcohol (2 L) was hydrogenated (50 bar) in an autoclave at 70 °C for 3 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated in vacuo and washed with n-pentane to afford the corresponding amine (150 g, 0.745 mol, 82%) as a crude material (white solid), which was used in the next step without further purification.

To a stirred solution of the crude amine (120 g, 0.596 mol) in THF (3 L) was added Et_3N (177 mL, 1.31 mol) at rt followed by the dropwise addition of triphosgene (62.0 g, 0.210 mol) in THF (0.5 L) at −10 °C, and the reaction mixture was stirred at the same temperature for 30 min. Then, the reaction mixture was stirred at rt for 2 h. The reaction was quenched with sat. $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography (silica gel, eluted with 50% EtOAc/petroleum ether) to afford **23a** (110 g, 0.484 mol, 81%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (9H, s), 2.46−2.53 (4H, m), 2.72 (1H, quin, J = 8.0 Hz), 4.34 (2H, s), 5.80 (1H, brs). LCMS (ESI/APCI) m/z 228.2 $[M + H]^+$.

*(2s,4s)-Ethyl 6-Oxo-7-oxa-5-azaspiro[3.4]octane-2-carboxylate (**23b**).* A mixture of **22b** (770 mg, 3.79 mmol) and 10% Pd-C (145 mg, 1.22 mmol) in MeOH (10 mL)/THF (10 mL) was hydrogenated under balloon pressure at rt for 72 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give (1s,3s)-ethyl 3-amino-3-(hydroxymethyl)cyclobutene-1-carboxylate (659 mg, 3.80 mmol, 100%) as a pale yellow oil. This product was subjected to the next reaction without further purification.

To a cooled (−10 °C) solution of the crude amine (656 mg, 3.79 mmol), DIPEA (1.45 mL, 8.33 mmol), and THF (8 mL) was added triphosgene (393 mg, 1.33 mmol), and the mixture was stirred at the same temperature for 30 min. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−90% EtOAc in hexane) to give **23b** (381 mg, 1.91 mmol, 2 steps 51% from **22b**) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (3H, t, J = 7.2 Hz), 2.47−2.64 (4H, m), 2.74−2.88 (1H, m), 4.17 (2H, q, J = 7.1 Hz), 4.37 (2H, s), 5.80 (1H, brs). LCMS (ESI/APCI) m/z 200.0 $[M + H]^+$.

*(2s,4s)-6-Oxo-7-oxa-5-azaspiro[3.4]octane-2-carboxylic Acid (**24**).* To **23a** (110 g, 0.484 mol) was added precooled TFA (1 L) at 0 °C, and the reaction mixture was stirred at rt for 2 h. After concentration in vacuo, the residue was azeotroped with toluene (×3) to give a crude compound. The crude compound was stirred with n-pentane, collected, and dried in vacuo to afford **24** (76.0 g,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

0.444 mol, 92%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.33−2.39 (4H, m), 2.73 (1H, quin, J = 9.08 Hz), 4.34 (2H, s), 8.09 (1H, s), 12.24 (1H, brs). LCMS (ESI/APCI) m/z 170.1 [M-H]$^-$.

*tert-Butyl 3-(3-Methoxy-3-oxopropyl)-3-nitroazetidine-1-carboxylate (**26**).* To a stirred solution of **25** (65 g, 0.32 mol) in MeOH (650 mL) were added methyl acrylate (34.7 mL, 0.385 mol) and K_2CO_3 (53.1 g, 0.385 mol) at 0 °C and the resultant reaction mixture was stirred at the same temperature for 3 h. After concentration in vacuo, the residue was quenched with sat. NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography (silica gel, eluted with 16−18% EtOAc/petroleum ether) to afford **26** (49 g, 0.17 mol, 53%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s), 2.34−2.38 (2H, m), 2.52−2.56 (2H, m), 3.70 (3H, s), 4.03 (2H, d, J = 10.80 Hz), 4.45 (2H, d, J = 10.00 Hz). LCMS (ESI/APCI) m/z 233.1 [M − t-Bu + H]$^+$.

*tert-Butyl-6-oxo-2,5-diazaspiro[3.4]octane-2-carboxylate (**27**).* To a stirred mixture of **26** (49 g, 0.17 mol) and $NiCl_2{\cdot}6H_2O$ (40.3 g, 0.169 mol) in MeOH (500 mL) was added $NaBH_4$ (32.1 g, 0.849 mol) at −10 °C, and the reaction mixture was stirred at the same temperature for 2 h. The reaction was quenched with aq. K_2CO_3 (100 mL), and the reaction mixture was stirred at rt for 2 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude compound was diluted with Et_2O and stirred for 1 h, collected, and dried in vacuo to afford **27** (29 g, 0.13 mol, 76%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s), 2.37−2.40 (4H, m), 4.00−4.02 (4H, m), 6.30 (1H, brs). LCMS (ESI/APCI) m/z 171.3 [M − t-Bu + H]$^+$.

*2,5-Diazaspiro[3.4]octan-6-one bis(4-methylbenzenesulfonate) (**28**).* A mixture of **27** (28 g, 0.12 mol) and p-TsOH-H_2O (51.8 g, 0.272 mol) in EtOAc (450 mL) was heated to 75 °C for 3 h. After being cooled to rt, the resulting precipitates were collected, washed with Et_2O, and dried in vacuo to afford **28** (52.5 g, 0.112 mol, 90%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.18−2.21 (2H, m), 2.29−2.35 (8H, m), 3.93−4.09 (4H, m), 7.13 (4H, d, J = 8.0 Hz), 7.49 (4H, d, J = 8.0 Hz), 8.28 (1H, brs), 8.46 (1H, brs), 8.83 (1H, brs). LCMS (ESI/APCI) m/z 127.2 [M + H]$^+$.

*tert-Butyl 3-(3-Methoxy-3-oxopropyl)-3-nitropiperidine-1-carboxylate (**30**).* To a cooled (0 °C) solution of **29** (1.09g, 4.73 mmol) and methyl acrylate (0.192 mL, 4.73 mmol) in MeOH (10 mL) was added K_2CO_3 (0.785 g, 5.68 mmol), and the mixture was stirred at rt for 4 h. The mixture was poured into sat. $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−60% EtOAc in hexane) to give **30** (0.923 g, 2.92 mmol, 62%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 1.40−1.49 (1H, m), 1.52−1.66 (1H, m), 1.77−1.93 (1H, m), 2.00−2.16 (2H, m), 2.23−2.42 (3H, m), 2.86−3.09 (1H, m), 3.32−3.43 (1H, m), 3.59 (3H, s), 3.59−3.69 (1H, m), 4.30 (1H, d, J = 14.2 Hz). LCMS (ESI/APCI) m/z 261.0 [M − t-Bu + H]$^+$.

*tert-Butyl 2-Oxo-1,7-diazaspiro[4.5]decane-7-carboxylate (**31**).* To a cooled (0 °C) mixture of **30** (923 mg, 2.92 mmol), $NiCl_2{\cdot}6H_2O$ (694 mg, 2.92 mmol), and MeOH (20 mL) was added $NaBH_4$ (552 mg, 14.59 mmol) portionwise. After being stirred at the same temperature for 2 h, the reaction was quenched with aq. K_2CO_3 [0.416 g in water (8 mL)]. The mixture was stirred at rt for 1.5 h and filtered through a Celite pad. The filtrate was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, filtered through a silica gel pad eluting with EtOAc/MeOH (4:1 v/v), and concentrated in vacuo to give **31** (603 mg, 2.37 mmol, 81%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (9H, s), 1.40−1.48 (1H, m), 1.53−1.74 (4H, m), 1.75−1.88 (1H, m), 2.12−2.25 (2H, m), 2.79−3.06 (2H, m), 3.38−3.65 (2H, m), 7.86 (1H, brs). LCMS (ESI/APCI) m/z 199.1 [M + H]$^+$.

*1,7-Diazaspiro[4.5]decan-2-one bis(4-methylbenzenesulfonate) (**32**).* A mixture of **31** (603 mg, 2.37 mmol) and p-TsOH-H_2O (992 mg, 5.22 mmol) in EtOAc (15 mL) was refluxed for 18 h. After being cooled to rt, the mixture was concentrated in vacuo, and the residual solid was collected, washed with EtOAc, and dried in vacuo to give **32** (1.17 g, 2.35 mmol, 99%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56−1.89 (4H, m), 1.97−2.10 (1H, m), 2.12−2.36 (8H, m), 2.80−3.19 (4H, m), 4.77−5.14 (1H, m), 7.12 (4H, d, J = 7.8 Hz), 7.48 (4H, d, J = 8.1 Hz), 7.75 (1H, s), 8.12-8.92 (2H, m). LCMS (ESI/APCI) m/z 155.1 [M + H]$^+$.

*tert-Butyl 4-((2-Chloro-4-fluorophenoxy)methyl)piperidine-1-carboxylate (**34**).* To a cooled (0 °C) solution of **33** (2.50 g, 11.6 mmol), 2-chloro-4-fluorophenol (1.49 mL, 13.9 mmol), and PBu_3 (3.47 mL, 13.9 mmol) in THF (50 mL) was added ADDP (3.52 g, 13.9 mmol), and the mixture was stirred at rt for 18 h. After filtration through a silica gel/NH silica gel pad, the filtrate was concentrated and purified by column chromatography (silica gel, eluted with 0−50% EtOAc in hexane) to give **34** (3.81 g, 11.1 mmol, 95%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21−1.38 (2H, m), 1.42−1.51 (9H, m), 1.78−2.11 (3H, m), 2.64−2.85 (2H, m), 3.75−3.97 (2H, m), 4.04−4.37 (2H, m), 6.80−7.00 (2H, m), 7.05−7.16 (1H, m). LCMS (ESI/APCI) m/z 288.1 [M − t-Bu + H]$^+$.

*4-((2-Chloro-4-fluorophenoxy)methyl)piperidine Hydrochloride (**35**).* To a solution of **34** (3.81 g, 11.1 mmol) in EtOAc (30 mL) was added 4 M HCl-EtOAc (22.2 mL, 88.7 mmol), and the mixture was stirred at rt for 14 h. The resultant precipitates were collected, washed with EtOAc, and dried in vacuo to give **35** (2.09 g, 7.46 mmol, 67%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.43−1.62 (2H, m), 1.84−1.98 (2H, m), 2.01−2.17 (1H, m), 2.83−2.97 (2H, m), 3.23−3.36 (2H, m), 3.93 (2H, d, J = 6.2 Hz), 7.13−7.26 (2H, m), 7.39−7.48 (1H, m), 8.81 (2H, brs). LCMS (ESI/APCI) m/z 244.1 [M + H]$^+$.

*tert-Butyl 4-((2-Chloro-4-fluorophenoxy)methyl)-4-methylpiperidine-1-carboxylate (**37**).* To a stirred mixture of **36** (1.00 g, 4.36 mmol) and 1-chloro-2,5-difluorobenzene (0.503 mL, 4.58 mmol) in THF (10 mL) was added t-BuOK (587 mg, 5.23 mmol) at 0 °C, and the mixture was stirred at rt overnight. The mixture was heated to reflux for 2 h, and then 1-chloro-2,5-difluorobenzene (0.503 mL, 4.58 mmol) and t-BuOK (587 mg, 5.23 mmol) were added to the mixture at rt. The mixture was heated to reflux for further 2 h, and then partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with EtOAc/hexane (0−15%) to give **37** (881 mg, 2.46 mmol, 57%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (3H, s), 1.46 (11H, s), 1.59−1.70 (2H, m), 3.21 (2H, ddd, J = 13.7, 9.9, 3.6 Hz), 3.64−3.81 (4H, m), 6.79−6.97 (2H, m), 7.12 (1H, dd, J = 7.9, 3.0 Hz). LCMS (ESI/APCI) m/z 302.1 [M − t-Bu + H]$^+$.

*4-((2-Chloro-4-fluorophenoxy)methyl)-4-methylpiperidine Hydrochloride (**38**).* A mixture of **37** (300 mg, 0.838 mmol) and 2 M HCl in MeOH (419 μL, 0.838 mmol) was stirred at rt overnight. The mixture was concentrated in vacuo to give **38** (226 mg, 0.768 mmol, 92%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.12 (3H, s), 1.49−1.68 (2H, m), 1.73−1.91 (2H, m), 3.01−3.23 (4H, m), 3.86 (2H, s), 7.20 (2H, dd, J = 6.8, 1.9 Hz), 7.45 (1H, dt, J = 8.5, 1.4 Hz), 8.35-8.85 (2H, m). LCMS (ESI/APCI) m/z 258.1 [M + H]$^+$.

*tert-Butyl 4-(1-(2-Chloro-4-fluorophenoxy)ethyl)piperidine-1-carboxylate (**40a**).* To a mixture of **39a** (3.02 g, 13.2 mmol) and Et_3N (3.67 mL, 26.3 mmol) in THF (30 mL) at 0 °C was added MsCl (1.22 mL, 15.8 mmol). After being stirred at rt for 3 days, MsCl (0.306 mL, 3.95 mmol) and Et_3N (1.83 mL, 13.2 mmol) were added thereto. The mixture was stirred for 3 days, and then MsCl (0.306 mL, 3.95 mmol) and Et_3N (1.83 mL, 13.2 mmol) were added. The mixture was stirred at rt for 6 h, and then EtOAc and water were added to the reaction mixture. The EtOAc layer was separated, washed with water and brine, dried over Na_2SO_4,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

concentrated in vacuo. The residue was dissolved in MeCN (30.0 mL), and Et_3N (1.83 mL, 13.2 mmol) and MsCl (0.612 mL, 7.90 mmol) were successively added at 0 °C. The mixture was stirred for 3 h. The mixture was diluted with sat. NH_4Cl, and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with EtOAc/hexane (50%) to give *tert*-butyl 4-(1-((methylsulfonyl)oxy)ethyl)piperidine-1-carboxylate (3.56 g, 11.6 mmol, 88%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18−1.35 (2H, m), 1.41 (3H, d, J = 6.4 Hz), 1.46 (9H, s), 1.61−1.84 (3H, m), 2.67 (2H, brs), 3.01 (3H, s), 4.09−4.35 (2H, m), 4.56−4.72 (1H, m).

The mesylate (0.617 g, 2.01 mmol), 2-chloro-4-fluorophenol (0.428 mL, 4.01 mmol), Cs_2CO_3 (1.31 g, 4.01 mmol), and DMF (7 mL) was stirred at 80 °C overnight. The mixture was diluted with EtOAc and water, and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−20% EtOAc in hexane) to **40a** (0.342 g, 0.956 mmol, 48%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21−1.42 (5H, m), 1.46 (9H, s), 1.66−1.99 (3H, m), 2.61−2.80 (2H, m), 4.04−4.29 (3H, m), 6.80−6.96 (2H, m), 7.11 (1H, dd, J = 8.2, 2.7 Hz). LCMS (ESI/APCI) m/z 302.0 $[M - t\text{-Bu} + H]^+$.

*tert-Butyl 3-((2-Chloro-4-fluorophenoxy)methyl)pyrrolidine-1-carboxylate (**40d**).* *p*-TsCl (837 mg, 4.39 mmol) was added to a solution of **39d** (803 mg, 3.99 mmol) in pyridine (10 mL) at 0 °C and the mixture was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with 1 M HCl, 10% K_2CO_3, and brine, and dried over Na_2SO_4. The solution was passed through NH silica gel and concentrated in vacuo to afford *tert*-butyl 3-((tosyloxy)methyl)pyrrolidine-1-carboxylate (1.38 g, 3.88 mmol, 97%) as a colorless oil. This was used in the next step without further purification.

A mixture of the tosylate (1.38 g, 3.88 mmol), 2-chloro-4-fluorophenol (0.508 mL, 4.66 mmol), and K_3PO_4 (1.24 g, 5.82 mmol) in DMF (10 mL) was heated at 80 °C for 2 h under N_2 atmosphere. The mixture was diluted with EtOAc and the solution was washed with water and brine, dried over Na_2SO_4. The solution was passed through NH silica gel, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4:1 to 1:1) to afford **40d** (1.11 g, 3.37 mmol, 87%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (9H, s), 1.63−1.82 (1H, m), 1.93−2.09 (1H, m), 2.55−2.69 (1H, m), 3.06−3.30 (2H, m), 3.33−3.50 (2H, m), 3.96−4.05 (2H, m), 7.15−7.21 (2H, m), 7.39−7.45 (1H, m).

*tert-Butyl 3-((2-Chloro-4-fluorophenoxy)methyl)azetidine-1-carboxylate (**40e**).* To a solution of **39e** (25.0 g, 134 mmol) in pyridine (40 mL) was added *p*-TsCl (28.0 g, 147 mmol) at rt. After being stirred overnight, the mixture was diluted with EtOAc (250 mL) and water (250 mL). The organic layer was washed with 0.5 M HCl (100 mL × 3) and brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo to give 43g of crude product as pale brown gum. A seed crystal was to the residue and settled overnight at rt. The resultant crystal was triturated with hexane to give *tert*-butyl 3-((tosyloxy)methyl)azetidine-1-carboxylate (28.0 g, 82.0 mmol, 61%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (2H, td, J = 12.3, 4.1 Hz), 1.39 (9H, s), 1.72 (2H, t, J = 10.9 Hz), 2.30 (1H, ddt, J = 11.9, 8.0, 4.2 Hz), 2.77 (2H, brs), 3.91−4.08 (2H, m), 5.85−5.99 (1H, m). LCMS (ESI/APCI) m/z 286.0 $[M - t\text{-Bu} + H]^+$.

The tosylate (2.33 g, 6.82 mmol) was added to a mixture of 2-chloro-4-fluorophenol (0.719 mL, 6.82 mmol) and K_2CO_3 (1.89 g, 13.7 mmol) in DMF (10 mL) at rt. The mixture was stirred at 80 °C for 7 h and then stirred at rt overnight. EtOAc and water were added to the reaction mixture at rt, then the organic layer was separated. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 5−26% EtOAc in hexane) to give **40e** (1.17 g, 3.69 mmol, 54%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 2.90−3.01 (1H, m), 3.73 (2H, brs), 3.88−4.00 (2H, m), 4.15 (2H, d, J = 6.0 Hz), 7.16−7.23 (2H, m), 7.40−7.46 (1H, m). LCMS (ESI/APCI) m/z 260.0 $[M - t\text{-Bu} + H]^+$.

*4-(1-(2-Chloro-4-fluorophenoxy)ethyl)piperidine 4-Methylbenzenesulfonate (**41a**).* A mixture of **40a** (0.340 g, 0.950 mmol) and *p*-TsOH-H_2O (0.207 g, 1.09 mmol) in EtOAc (4 mL) was refluxed for 2 h. After being cooled to rt, the mixture was concentrated in vacuo, and the residual semisolid was triturated with EtOAc/heptane to give **41a** (0.376 g, 0.875 mmol, 92%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (3H, d, J = 6.1 Hz), 1.39−1.64 (2H, m), 1.77−2.05 (3H, m), 2.29 (3H, s), 2.78−3.01 (2H, m), 3.24−3.45 (2H, m), 4.30−4.45 (1H, m), 7.05−7.29 (4H, m), 7.37−7.55 (3H, m), 8.17 (1H, brs), 8.48 (1H, brs). LCMS (ESI/APCI) m/z 258.0 $[M + H]^+$.

*4-(1-(2-Chloro-4-fluorophenoxy)propyl)piperidine (**41b**).* MsCl (2.00 mL, 25.9 mmol) was added to a solution of **39b** (1.80 g, 7.40 mmol) and Et_3N (5.15 mL, 37.0 mmol) in MeCN (30 mL) at 0 °C. The mixture was stirred at rt over weekend. MsCl (2.00 mL, 25.9 mmol) and Et_3N (5.15 mL, 37.0 mmol) was added to the mixture and the resulting mixture was stirred at rt overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 25−50% EtOAc in hexane) to give *tert*-butyl 4-(1-((methylsulfonyl)oxy)propyl)piperidine-1-carboxylate (1.37 g, 4.26 mmol, 58%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (3H, t, J = 7.4 Hz), 1.02−1.25 (2H, m), 1.39 (9H, s), 1.50−1.92 (5H, m), 2.67 (2H, brs), 3.16 (3H, s), 3.90−4.06 (2H, m), 4.45 (1H, q, J = 5.7 Hz). LCMS (ESI/APCI) m/z 266.2 $[M - t\text{-Bu} + H]^+$.

A mixture of the mesylate (650 mg, 2.02 mmol), 2-chloro-4-fluorophenol (0.432 mL, 4.04 mmol), Cs_2CO_3 (1.32 g, 4.04 mmol), and DMF (30 mL) was stirred at 80 °C overnight. The mixture was diluted with EtOAc and water, and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−20% EtOAc in hexane, inject column: NH to remove the phenol) to give **40b**. This was dissolved in EtOAc (10 mL) and 4 M HCl in EtOAc (10 mL) was added. The mixture was stirred at rt for 2 h. The mixture was concentrated, and the residue was purified by column chromatography (NH silica gel, eluted with 0−25% MeOH in EtOAc) to give **41b** (235 mg, 0.865 mmol, 43%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 7.4 Hz), 1.11−1.30 (2H, m), 1.45−1.75 (5H, m), 1.91 (1H, brs), 2.40 (2H, td, J = 12.1, 2.3 Hz), 2.86−2.99 (2H, m), 4.12−4.24 (1H, m), 7.07−7.25 (2H, m), 7.38 (1H, dd, J = 8.3, 3.0 Hz). LCMS (ESI/APCI) m/z 272.1 $[M + H]^+$.

*4-(1-(Chloro-4-fluorophenoxy)-2,2,2-trifluoroethyl)piperidine (**41c**).* Tf_2O (2.00 mL, 11.9 mmol) was added to a solution of **39c** (2.24 g, 7.91 mmol) in pyridine (25 mL) at 0 °C. The mixture was stirred at rt overnight. The mixture was concentrated in vacuo, and the residue was dissolved in EtOAc and 1 M HCl. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−50% EtOAc in hexane) to give *tert*-butyl 4-(2,2,2-trifluoro-1-(((trifluoromethyl)sulfonyl)-oxy)ethyl)piperidine-1-carboxylate (3.00 g, 7.22 mmol, 91%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (2H, td, J = 12.3, 4.1 Hz), 1.39 (9H, s), 1.72 (2H, t, J = 10.9 Hz), 2.30 (1H, ddt, J = 11.9, 8.0, 4.2 Hz), 2.77 (2H, brs), 3.91−4.08 (2H, m), 5.85−5.99 (1H, m). LCMS (ESI/APCI) m/z 360.0 $[M - t\text{-Bu} + H]^+$.

A mixture of the mesylate (1.45 g, 3.49 mmol), 2-chloro-4-fluorophenol (0.745 mL, 6.98 mmol), Cs_2CO_3 (2.28 g, 6.98 mmol), and DMF (20 mL) was stirred at 80 °C overnight. The mixture was diluted with EtOAc and water, and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−20% EtOAc in hexane,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

inject column: NH to remove the phenol) to give **40c**. This was dissolved in EtOAc (10 mL) and 4 M HCl in EtOAc (10 mL) was added. The mixture was stirred at rt for 2 h. The mixture was concentrated in vacuo, and the residue was purified by column chromatography (NH silica gel, eluted with 0−25% MeOH in EtOAc) to give **41c** (0.172 g, 0.552 mmol, 16%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22−1.52 (2H, m), 1.57−1.73 (2H, m), 1.83−2.27 (2H, m), 2.35−2.49 (2H, m), 2.84−3.03 (2H, m), 5.02−5.19 (1H, m), 7.22 (1H, ddd, J = 9.3, 8.1, 3.0 Hz), 7.35−7.54 (2H, m). LCMS (ESI/APCI) m/z 312.2 $[M + H]^+$.

*3-((2-Chloro-4-fluorophenoxy)methyl)pyrrolidine 4-Methylbenzenesulfonate (**41d**).* A mixture of **40d** (1.11 g, 3.37 mmol) and p-TsOH-H_2O (0.704 g, 3.70 mmol) in EtOAc (20 mL) was refluxed for 2 h and cooled to rt. The resultant precipitate was collected, washed with EtOAc, and dried in vacuo to give **41d** (0.983 g, 2.45 mmol, 73%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70−1.86 (1H, m), 2.02−2.18 (1H, m), 2.29 (3H, s), 2.65−2.83 (1H, m), 2.98−3.45 (4H, m), 3.97−4.15 (2H, m), 7.12 (2H, d, J = 7.7 Hz), 7.16−7.25 (2H, m), 7.41−7.53 (3H, m), 8.62-8.80 (2H, m).

*3-((2-Chloro-4-fluorophenoxy)methyl)azetidine 4-Methylbenzenesulfonate (**41e**).* p-TsOH-H_2O (0.769 g, 4.04 mmol) was added to a mixture of **40e** (1.16 g, 3.67 mmol) in EtOAc (25 mL) at rt. The mixture was stirred at 70 °C for 2 h and cooled at rt. The resulting solid was collected, washed with EtOAc, and dried in vacuo to give **41e** (1.21 g, 3.11 mmol, 85%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.86−3.97 (2H, m), 3.99−4.10 (2H, m), 4.18 (2H, d, J = 5.9 Hz), 7.11 (2H, d, J = 7.9 Hz), 7.19−7.24 (2H, m), 7.44−7.51 (3H, m), 8.60 (2H, brs).

*tert-Butyl 4-((2-Chloro-4-fluorobenzyl)oxy)piperidine-1-carboxylate (**43a**).* To a solution of **42a** (1.00 g, 4.97 mmol) in DMF (15 mL) was added 60% NaH (0.238 g, 5.96 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. Then, 1-(bromomethyl)-2-chloro-4-fluorobenzene (1.22 g, 5.47 mmol) was added dropwise to the reaction mixture and the resulting white suspension was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with water and brine, dried over Na_2SO_4. The solution was passed through NH silica gel, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3:1 to 1:1) to afford **43a** (1.34 g, 3.90 mmol, 78%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35−1.49 (11H, s), 1.78−1.89 (2H, m), 3.00−3.13 (2H, m), 3.57−3.68 (3H, m), 4.55 (2H, s), 7.23 (1H, td, J = 8.6, 2.4 Hz), 7.43 (1H, dd, J = 8.9, 2.4 Hz), 7.56 (1H, dd, J = 8.7, 6.4 Hz).

*tert-Butyl 3-((2-Chloro-4-fluorobenzyl)oxy)pyrrolidine-1-carboxylate (**43b**).* To a solution of **43a** (1.00 g, 5.34 mmol) in DMF (15 mL) was added 60% NaH (0.256 g, 6.41 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. Then, 1-(bromomethyl)-2-chloro-4-fluorobenzene (1.31 g, 5.87 mmol) was added dropwise to the reaction mixture and the resulting white suspension was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with water and brine, dried over Na_2SO_4. The solution was passed through NH silica gel, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3:1 to 1:1) to afford **43b** (1.76 g, 5.34 mmol, 100%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (9H, s), 1.88−2.03 (2H, s), 3.21−3.39 (4H, m), 4.12−4.21 (1H, m), 4.52 (2H, s), 7.22 (1H, td, J = 8.5, 2.6 Hz), 7.44 (1H, dd, J = 9.0, 2.6 Hz), 7.53 (1H, dd, J = 8.7, 6.4 Hz).

*tert-Butyl 3-((2-Chloro-4-fluorobenzyl)oxy)azetidine-1-carboxylate (**43c**).* To a solution of **43c** (1.00 g, 5.77 mmol) in DMF (15 mL) was added 60% NaH (0.277 g, 6.93 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. Then, 1-(bromomethyl)-2-chloro-4-fluorobenzene (1.42 g, 6.35 mmol) was added dropwise to the reaction mixture and the resulting white suspension was stirred at rt for 16 h. The mixture was diluted with EtOAc and the solution was washed with water and brine, dried over Na_2SO_4. The solution was passed through NH silica gel, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 3:1 to 1:1) to afford **43c** (1.82 g, 5.76 mmol, 100%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 3.66−3.74 (2H, m), 3.98−4.07 (2H, m), 4.33−4.41 (1H, m), 4.48 (2H, s), 7.24 (1H, td, J = 8.5, 2.6 Hz), 7.46 (1H, dd, J = 9.0, 2.6 Hz), 7.57 (1H, dd, J = 8.5, 6.2 Hz).

*4-((2-Chloro-4-fluorobenzyl)oxy)piperidine 4-Methylbenzenesulfonate (**44a**).* A mixture of **43a** (1.70 g, 4.94 mmol) and p-TsOH-H_2O (1.04 g, 5.44 mmol) in EtOAc (30 mL) was refluxed for 3 h and cooled to rt. The resultant precipitate was collected, washed with EtOAc, and dried in vacuo to give **44a** (0.520 g, 1.25 mmol, 25%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65−1.82 (2H, m), 1.91−2.06 (2H, m), 2.29 (3H, s), 2.93−3.07 (2H, m), 3.12−3.25 (2H, m), 3.66−3.79 (1H, m), 4.55 (2H, s), 7.11 (2H, d, J = 7.9 Hz), 7.20−7.29 (1H, m), 7.42−7.51 (3H, m), 7.57 (1H, dd, J = 8.7, 6.4 Hz), 8.32 (2H, br s). LCMS (ESI/APCI) m/z 244.0 $[M + H]^+$.

*3-((2-Chloro-4-fluorobenzyl)oxy)pyrrolidine 4-Methylbenzenesulfonate (**44b**).* A mixture of **43b** (1.76 g, 5.34 mmol) and p-TsOH-H_2O (1.12 g, 5.87 mmol) in EtOAc (30 mL) was refluxed for 3 h and cooled to rt. The resultant precipitate was collected, washed with EtOAc, and dried in vacuo to give **44b** (1.64 g, 4.08 mmol, 76%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65−1.82 (2H, m), 1.90−2.07 (1H, m), 2.09−2.24 (1H, m), 2.29 (3H, s), 3.10−3.42 (4H, m), 4.30−4.38 (1H, m), 4.49−4.61 (2H, m), 7.11 (2H, d, J = 7.9 Hz), 7.21−7.31 (1H, m), 7.43−7.52 (3H, m), 7.58 (1H, dd, J = 8.5, 6.2 Hz), 8.81 (2H, br s). LCMS (ESI/APCI) m/z 230.0 $[M + H]^+$.

*3-((2-Chloro-4-fluorobenzyl)oxy)azetidine 4-Methylbenzenesulfonate (**44c**).* A mixture of **43c** (1.82 g, 5.76 mmol) and p-TsOH-H_2O (1.21 g, 6.34 mmol) in EtOAc (30 mL) was refluxed for 3 h and cooled to rt. The resultant precipitate was collected, washed with EtOAc, and dried in vacuo to give **44c** (1.64 g, 4.23 mmol, 73%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.82−3.92 (2H, m), 4.12−4.22 (2H, m), 4.43−4.56 (3H, m), 7.11 (2H, d, J = 7.9 Hz), 7.22−7.33 (1H, m), 7.44−7.53 (3H, m), 7.59 (1H, dd, J = 8.7, 6.4 Hz), 8.65 (2H, br s). LCMS (ESI/APCI) m/z 216.0 $[M + H]^+$.

*3-((2-Chloro-4-methylbenzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46a**).* To a solution of **45a** (0.783 g, 5.00 mmol) and Et_3N (1.53 mL, 11.0 mmol) in THF (16.7 mL) was added MsCl (0.774 mL, 10.0 mmol) dropwise at 0 °C. The resultant pale yellow suspension was stirred at rt for 3 h. The mixture was partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered through a short pad of silica gel, and concentrated in vacuo to give 2-chloro-4-methylbenzyl methanesulfonate (1.17 g, 4.99 mmol, 100%), which was used in the next step without further purification.

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (0.786 g, 4.54 mmol) in DMF (8.51 mL) was added 60% NaH (0.218 g, 5.45 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of 2-chloro-4-methylbenzyl methanesulfonate (1.17 g, 4.99 mmol) in DMF (2.84 mL) was added dropwise to the reaction mixture. The pale yellow suspension was stirred at rt for 38 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((2-chloro-4-methylbenzyl)oxy)azetidine-1-carboxylate (1.32 g, 4.23 mmol, 93%) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 2.30 (3H, s), 3.68 (2H, dd, J = 8.8, 3.3 Hz), 4.01 (2H, dd, J = 9.1, 6.8 Hz), 4.35 (1H, tt, J = 6.4, 4.0 Hz), 4.46 (2H, s), 7.16 (1H, dd, J = 7.7, 0.9 Hz), 7.29 (1H, s), 7.39 (1H, d, J = 7.7 Hz).

A solution of *tert*-butyl 3-((2-chloro-4-methylbenzyl)oxy)-azetidine-1-carboxylate (1.32 g, 4.23 mmol) and p-TsOH-H_2O (0.886 g, 4.66 mmol) in EtOAc (21.2 mL) was refluxed for 2 h. After being cooled to rt, the mixture was diluted with hexane to afford white precipitates. The resultant precipitates were collected, washed with EtOAc/hexane and dried in vacuo to give **46a** (1.12 g,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

2.92 mmol, 69%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21−2.36 (6H, m), 3.85 (2H, dd, J = 12.1, 5.3 Hz), 4.15 (2H, dd, J = 12.1, 6.6 Hz), 4.39−4.56 (3H, m), 7.11 (2H, d, J = 7.7 Hz), 7.18 (1H, d, J = 7.0 Hz), 7.31 (1H, s), 7.41 (1H, d, J = 7.7 Hz), 7.44−7.50 (2H, m), 8.60 (2H, brs). LCMS (ESI/APCI) m/z 212.1 $[M + H]^+$.

*3-((3-Chloro-4-(trifluoromethyl)benzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46b**).* To a solution of **45b** (1.84 g, 8.74 mmol) and Et_3N (2.82 mL, 20.2 mmol) in THF (30.7 mL) was added MsCl (1.43 mL, 18.5 mmol) dropwise at 0 °C. The resultant pale yellow suspension was stirred at rt for 3 h. The mixture was partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered through a short pad of silica gel, and concentrated in vacuo to give 3-chloro-4-(trifluoromethyl)benzyl methanesulfonate (2.53 g, 8.76 mmol, 100%), which was used in the next step without further purification.

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (1.45 g, 8.37 mmol) in DMF (15.7 mL) was added 60% NaH (0.402 g, 10.1 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of 3-chloro-4-(trifluoromethyl)benzyl methanesulfonate (2.53 g, 8.76 mmol) in DMF (5.23 mL) was added dropwise to the reaction mixture. The pale yellow suspension was stirred at rt for 3 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((3-chloro-4-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (2.66 g, 7.27 mmol, 87%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 3.73 (2H, dd, J = 9.4, 3.4 Hz), 3.94−4.10 (2H, m), 4.30−4.41 (1H, m), 4.53 (2H, s), 7.52 (1H, d, J = 7.6 Hz), 7.69 (1H, s), 7.85 (1H, d, J = 8.1 Hz).

A solution of *tert*-butyl 3-((3-chloro-4-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (340 mg, 0.930 mmol) and *p*-TsOH-H_2O (194 mg, 1.02 mmol) in EtOAc (4.65 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **46b** (351 mg, 0.802 mmol, 86%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.89 (2H, dd, J = 12.1, 4.9 Hz), 4.10−4.22 (2H, m), 4.38−4.52 (1H, m), 4.58 (2H, s), 7.11 (2H, d, J = 7.7 Hz), 7.42−7.49 (2H, m), 7.53 (1H, d, J = 8.1 Hz), 7.73 (1H, s), 7.88 (1H, d, J = 8.3 Hz), 8.58 (2H, brs). LCMS (ESI/APCI) m/z 266.1 $[M + H]^+$.

*3-((4-Methyl-3-(trifluoromethyl)benzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46c**).* To a solution of **45c** (1.89 g, 9.94 mmol) and Et_3N (3.05 mL, 21.9 mmol) in THF (33.1 mL) was added MsCl (1.54 mL, 19.9 mmol) dropwise at 0 °C. The resultant pale yellow suspension was stirred at rt for 30 min. The mixture was partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered through a short pad of silica gel, and concentrated in vacuo to give 4-methyl-3-(trifluoromethyl)benzyl methanesulfonate (2.73 g, 10.2 mmol, quant.), which was used in the next step without further purification.

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (1.57 g, 9.04 mmol) in DMF (17.0 mL) was added 60% NaH (0.434 g, 10.9 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of 4-methyl-3-(trifluoromethyl)benzyl methanesulfonate (2.67 g, 9.94 mmol) in DMF (5.65 mL) was added dropwise to the reaction mixture. The pale yellow suspension was stirred at rt for 3 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((4-methyl-3-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (2.86 g, 8.28 mmol, 92%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 2.44 (3H, d, J = 1.7 Hz), 3.69 (2H, dd, J = 9.0, 3.3 Hz), 3.95−4.09 (2H, m), 4.28−4.38 (1H, m), 4.46 (2H, s), 7.39−7.47 (1H, m), 7.49−7.57 (1H, m), 7.63 (1H, s).

A solution of *tert*-butyl 3-((4-methyl-3-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (2.86 g, 8.28 mmol) and *p*-TsOH-H_2O (1.73 g, 9.11 mmol) in EtOAc (41.4 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **46c** (3.05 g, 7.31 mmol, 88%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 2.44 (3H, d, J = 1.7 Hz), 3.81−3.91 (2H, m), 4.09−4.19 (2H, m), 4.44 (1H, tt, J = 6.5, 5.2 Hz), 4.52 (2H, s), 7.11 (2H, d, J = 8.3 Hz), 7.42−7.50 (3H, m), 7.51−7.58 (1H, m), 7.66 (1H, s), 8.59 (2H, brs). LCMS (ESI/APCI) m/z 246.1 $[M + H]^+$.

*3-((4-Chloro-3-methylbenzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46d**).* To a solution of **45d** (1.40 g, 8.94 mmol) and Et_3N (2.74 mL, 19.7 mmol) in THF (29.8 mL) was added MsCl (1.38 mL, 17.9 mmol) dropwise at 0 °C. The resultant pale-orange suspension was stirred at rt for 16 h. The mixture was partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered through a short pad of silica gel, and concentrated in vacuo to give 4-chloro-3-methylbenzyl methanesulfonate (2.20 g, 9.37 mmol, quant.), which was used in the next step without further purification.

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (1.41 g, 8.13 mmol) in DMF (15.2 mL) was added 60% NaH (0.390 g, 9.76 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of 4-chloro-3-methylbenzyl methanesulfonate (2.10 g, 8.94 mmol) in DMF (5.08 mL) was added dropwise to the reaction mixture. The pale yellow suspension was stirred at rt for 3 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((4-chloro-3-methylbenzyl)oxy)azetidine-1-carboxylate (2.21 g, 7.09 mmol, 87%) as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 2.33 (3H, s), 3.60−3.75 (2H, m), 3.94−4.08 (2H, m), 4.31 (1H, ddd, J = 6.4, 4.1, 2.4 Hz), 4.38 (2H, s), 7.18 (1H, dd, J = 8.1, 1.9 Hz), 7.33 (1H, d, J = 1.7 Hz), 7.38 (1H, d, J = 8.1 Hz).

A solution of *tert*-butyl 3-((4-chloro-3-methylbenzyl)oxy)-azetidine-1-carboxylate (2.21 g, 7.09 mmol) and *p*-TsOH-H_2O (1.48 g, 7.80 mmol) in EtOAc (35.4 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **46d** (2.37 g, 6.17 mmol, 87%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 2.33 (3H, s), 3.83 (2H, dd, J = 12.1, 5.3 Hz), 4.05−4.19 (2H, m), 4.34−4.50 (3H, m), 7.11 (2H, d, J = 7.7 Hz), 7.20 (1H, dd, J = 8.2, 1.8 Hz), 7.33 (1H, s), 7.41 (1H, d, J = 8.1 Hz), 7.44−7.51 (2H, m), 8.57 (2H, brs). LCMS (ESI/APCI) m/z 212.1 $[M + H]^+$.

*3-((3-Chloro-4-methylbenzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46e**).* To a stirred solution of **45e** (15.5 g, 99.0 mmol) in DME (200 mL) was added dropwise phosphorus tribromide (5.58 mL, 59.4 mmol) at 0 °C, and the mixture was stirred at rt for 3 h. The reaction was quenched with ice water and then extracted with EtOAc/hexane. The extract was washed with water, aq. $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−40% EtOAc in hexane) to give 4-(bromomethyl)-2-chloro-1-methylbenzene (21.7 g, 99.0 mmol, 100%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.32 (3H, s), 4.68 (2H, s), 7.27−7.40 (2H, m), 7.45−7.58 (1H, m).

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (8.89 g, 51.3 mmol) in DMF (96 mL) was added 60% NaH (2.46 g, 61.6 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min. Then, a solution of 4-(bromomethyl)-2-chloro-1-methylbenzene (12.4 g, 56.4 mmol) in DMF (32.1 mL) was added dropwise to the reaction mixture. The white suspension was stirred at rt for 16 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

((3-chloro-4-methylbenzyl)oxy)azetidine-1-carboxylate (15.9 g, 50.8 mmol, 99%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 2.32 (3H, s), 3.67 (2H, dd, J = 9.1, 3.8 Hz), 3.92−4.09 (2H, m), 4.25−4.35 (1H, m), 4.39 (2H, s), 7.21 (1H, dd, J = 8.0, 1.5 Hz), 7.33 (1H, d, J = 8.0 Hz), 7.39 (1H, d, J = 1.5 Hz).

A solution of *tert*-butyl 3-((3-chloro-4-methylbenzyl)oxy)-azetidine-1-carboxylate (39.3 g, 126 mmol) and *p*-TsOH-H_2O (26.4 g, 139 mmol) in EtOAc (420 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **46e** (44.8 g, 117 mmol, 92%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 2.32 (3H, s), 3.84 (2H, dd, J = 11.5, 4.7 Hz), 4.13 (2H, dd, J = 11.7, 6.8 Hz), 4.34−4.50 (3H, m), 7.11 (2H, d, J = 8.0 Hz), 7.22 (1H, dd, J = 7.6, 1.5 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.42 (1H, d, J = 1.5 Hz), 7.45−7.52 (2H, m), 8.58 (2H, brs). LCMS (ESI/APCI) m/z 212.1 $[M + H]^+$.

*3-((4-Chloro-3-(trifluoromethyl)benzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46f**).* To a stirred solution of **45f** (2.08 g, 9.88 mmol) in DME (25 mL) was added dropwise phosphorus tribromide (0.557 mL, 5.93 mmol) at 0 °C, and the mixture was stirred at rt for 2.5 h. The reaction was quenched with ice water and then extracted with EtOAc/hexane. The extract was washed with water, aq. $NaHCO_3$, and brine, dried over Na_2SO_4, filtered through a silica gel/NH silica gel pad, and concentrated in vacuo to give 4-(bromomethyl)-1-chloro-2-(trifluoromethyl)benzene (2.33 g, 8.50 mmol, 86%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.47 (2H, s), 7.43−7.59 (2H, m), 7.71 (1H, s).

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (0.950 g, 5.48 mmol) in DMF (12 mL) was added 60% NaH (0.285 g, 7.13 mmol) at 0 °C and the mixture was stirred at the same temperature for 30 min. Then, a solution of 4-(bromomethyl)-1-chloro-2-(trifluoromethyl)benzene (1.50 g, 5.48 mmol) in DMF (6 mL) was added dropwise to the reaction mixture at 0 °C and the mixture was stirred at rt overnight. The mixture was quenched with ice water and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−33% EtOAc in hexane) to give *tert*-butyl 3-((4-chloro-3-(trifluoromethyl)benzyl)oxy)azetidine-1-carboxylate (1.78 g, 4.86 mmol, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (9H, s), 3.83−3.93 (2H, m), 4.05−4.16 (2H, m), 4.33 (1H, tt, J = 6.4, 4.3 Hz), 4.45 (2H, s), 7.40−7.54 (2H, m), 7.65 (1H, s).

A mixture of *tert*-butyl 3-((4-chloro-3-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (1.77 g, 4.85 mmol), *p*-TsOH-H_2O (0.969 g, 5.09 mmol), and EtOAc (16 mL) was refluxed for 3 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **46f** (1.77 g, 4.04 mmol, 83%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.82−3.96 (2H, m), 4.11−4.21 (2H, m), 4.39−4.51 (1H, m), 4.56 (2H, s), 7.11 (2H, d, J = 7.9 Hz), 7.41−7.55 (2H, m), 7.61−7.79 (2H, m), 7.85 (1H, d, J = 1.5 Hz), 8.62 (2H, brs). LCMS (ESI/APCI) m/z 266.1 $[M + H]^+$.

*3-((3-Methyl-4-(trifluoromethyl)benzyl)oxy)azetidine 4-Methylbenzenesulfonate (**46g**).* To a mixture of *tert*-butyl 3-((3-chloro-4-(trifluoromethyl)benzyl)oxy)azetidine-1-carboxylate (500 mg, 1.37 mmol), trimethylboroxine (191 μL, 1.37 mmol), SPhos (56.1 mg, 0.140 mmol) and K_3PO_4 (870 mg, 4.10 mmol) in toluene (6.15 mL) and water (0.683 mL) was added $Pd(OAc)_2$ (15.3 mg, 70.0 μmol). The mixture was stirred at 100 °C for 16 h. After being cooled to rt, the mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((3-methyl-4-(trifluoromethyl)benzyl)oxy)-azetidine-1-carboxylate (299 mg, 0.866 mmol, 63%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 2.44 (3H, d, J = 1.7 Hz), 3.71 (2H, dd, J = 9.1, 3.2 Hz), 3.97−4.09 (2H, m), 4.30−4.39 (1H, m), 4.48 (2H, s), 7.35 (1H, d, J = 8.1 Hz), 7.40 (1H, s), 7.65 (1H, d, J = 8.1 Hz).

A solution of *tert*-butyl 3-((3-methyl-4-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (299 mg, 0.866 mmol) and *p*-TsOH-H_2O (181 mg, 0.950 mmol) in EtOAc (4.33 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc/hexane, and dried in vacuo to give **46g** (268 mg, 0.642 mmol, 74%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 2.45 (3H, d, J = 1.7 Hz), 3.80−3.94 (2H, m), 4.09−4.22 (2H, m), 4.45 (1H, quin, J = 5.9 Hz), 4.53 (2H, s), 7.11 (2H, d, J = 7.9 Hz), 7.36 (1H, d, J = 7.7 Hz), 7.39−7.44 (1H, m), 7.45−7.51 (2H, m), 7.67 (1H, d, J = 7.9 Hz), 8.26-8.98 (2H, m). LCMS (ESI/APCI) m/z 246.1 $[M + H]^+$.

*3-((2,4-Dichlorobenzyl)oxy)azetidine 4-Methylbenzenesulfonate (**48a**).* To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (300 mg, 1.73 mmol) in DMF (3.25 mL) was added 60% NaH (83 mg, 2.1 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of **47a** (372 mg, 1.91 mmol) in DMF (1.08 mL) was added dropwise to the reaction mixture. The white suspension was stirred at rt for 16 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((2,4-dichlorobenzyl)-oxy)azetidine-1-carboxylate (540 mg, 1.63 mmol, 94%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 3.71 (2H, dd, J = 8.9, 3.2 Hz), 3.95−4.09 (2H, m), 4.33−4.43 (1H, m), 4.49 (2H, s), 7.43−7.48 (1H, m), 7.52−7.58 (1H, m), 7.64 (1H, d, J = 1.9 Hz).

A solution of *tert*-butyl 3-((2,4-dichlorobenzyl)oxy)azetidine-1-carboxylate (540 mg, 1.63 mmol) and *p*-TsOH-H_2O (340 mg, 1.79 mmol) in EtOAc (8.13 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **48a** (564 mg, 1.40 mmol, 86%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.88 (2H, dd, J = 12.1, 5.1 Hz), 4.17 (2H, dd, J = 12.3, 6.6 Hz), 4.40−4.53 (1H, m), 4.55 (2H, s), 7.11 (2H, d, J = 8.5 Hz), 7.43−7.51 (3H, m), 7.53−7.59 (1H, m), 7.66 (1H, d, J = 2.1 Hz), 8.63 (2H, brs). LCMS (ESI/APCI) m/z 232.1 $[M + H]^+$.

*3-((2-Chloro-4-(trifluoromethyl)benzyl)oxy)azetidine 4-Methylbenzenesulfonate (**48b**).* To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (0.600 g, 3.46 mmol) in DMF (6 mL) was added 60% NaH (0.180 g, 4.50 mmol) at 0 °C and the mixture was stirred at the same temperature for 10 min. Then, a solution of **47b** (0.873 g, 3.81 mmol) in DMF (2 mL) was added dropwise to the reaction mixture at 0 °C and the mixture was stirred at rt overnight. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−25% EtOAc in hexane) to give *tert*-butyl 3-((2-chloro-4-(trifluoromethyl)-benzyl)oxy)azetidine-1-carboxylate (1.01 g, 2.75 mmol, 79%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (9H, s), 3.93 (2H, dd, J = 9.8, 4.5 Hz), 4.09−4.21 (2H, m), 4.39 (1H, tt, J = 6.4, 4.3 Hz), 4.57 (2H, s), 7.51−7.58 (1H, m), 7.60−7.71 (2H, m).

A mixture of *tert*-butyl 3-((2-chloro-4-(trifluoromethyl)benzyl)-oxy)azetidine-1-carboxylate (1.00 g, 2.74 mmol), *p*-TsOH-H_2O (0.548 g, 2.88 mmol), and EtOAc (10 mL) was refluxed for 3 h. After being cooled to rt, the mixture was stirred for 1 h. Then, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **48b** (1.01 g, 2.31 mmol, 84%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.92 (2H, dd, J = 12.1, 5.3 Hz), 4.19 (2H, dd, J = 12.1, 6.4 Hz), 4.48−4.60 (1H, m), 4.65 (2H, s), 7.11 (2H, d, J = 7.9 Hz), 7.47 (2H, d, J = 8.3 Hz), 7.74−7.85 (2H, m), 7.91−7.97 (1H, m), 8.62 (2H, brs). LCMS (ESI/APCI) m/z 266.0 $[M + H]^+$.

*3-((3,4-Dichlorobenzyl)oxy)azetidine 4-Methylbenzenesulfonate (**48c**).* To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (300 mg, 1.73 mmol) in DMF (3.25 mL) was added 60% NaH (83 mg, 2.1 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of **47c** (372 mg, 1.91 mmol) in DMF (1.08 mL) was added dropwise to the reaction

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

mixture. The yellow suspension was stirred at rt for 16 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((3,4-dichlorobenzyl)-oxy)azetidine-1-carboxylate (527 mg, 1.59 mmol, 92%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (9H, s), 3.70 (2H, dd, J = 9.1, 3.2 Hz), 3.95−4.08 (2H, m), 4.28−4.37 (1H, m), 4.43 (2H, s), 7.35 (1H, dd, J = 8.2, 2.0 Hz), 7.58−7.66 (2H, m).

A solution of *tert*-butyl 3-((3,4-dichlorobenzyl)oxy)azetidine-1-carboxylate (527 mg, 1.59 mmol) and *p*-TsOH-H_2O (332 mg, 1.74 mmol) in EtOAc (7.93 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **48c** (547 mg, 1.35 mmol, 85%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.86 (2H, dd, J = 12.2, 5.0 Hz), 4.15 (2H, dd, J = 12.1, 6.6 Hz), 4.38−4.46 (1H, m), 4.48 (2H, s), 7.11 (2H, d, J = 7.7 Hz), 7.36 (1H, dd, J = 8.2, 2.0 Hz), 7.44−7.51 (2H, m), 7.62−7.69 (2H, m), 8.60 (2H, brs). LCMS (ESI/APCI) m/z 232.1 $[M + H]^+$.

*3-((3,4-Bis(trifluoromethyl)benzyl)oxy)azetidine 4-Methylbenzenesulfonate (**50**).* A solution of **49** (1.00 g, 3.87 mmol) in THF (12.9 mL) was cooled to 0 °C. BH_3−THF (7.75 mL, 7.75 mmol, 1.0 M) was added thereto dropwise over 3 min at the same temperature. The resultant mixture was stirred at rt for 18 h. The reaction was quenched with MeOH at 0 °C and the mixture was stirred at rt for 1 h. After removal of organic solvents in vacuo, the mixture was partitioned between water and EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered through a short pad of silica gel/NH silica gel (eluting with EtOAc), and concentrated in vacuo to give (3,4-bis(trifluoromethyl)phenyl)-methanol (0.945 g, 3.87 mmol, 100%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.68 (2H, d, J = 5.7 Hz), 5.61 (1H, t, J = 5.7 Hz), 7.84 (1H, d, J = 8.1 Hz), 7.96 (1H, s), 8.02 (1H, d, J = 8.1 Hz).

To a solution of (3,4-bis(trifluoromethyl)phenyl)methanol (0.945 g, 3.87 mmol) and Et_3N (1.19 mL, 8.52 mmol) in THF (12.9 mL) was added MsCl (0.599 mL, 7.74 mmol) dropwise at 0 °C. The resultant pale yellow suspension was stirred at rt for 2 h. The mixture was partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered through a short pad of silica gel, and concentrated in vacuo to give 3,4-bis(trifluoromethyl)benzyl methanesulfonate as a crude material (1.32 g, quant.), which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.33 (3H, s), 5.48 (2H, s), 8.00 (1H, d, J = 8.1 Hz), 8.09-8.18 (2H, m).

To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (0.610 g, 3.52 mmol) in DMF (6.60 mL) was added 60% NaH (0.169 g, 4.22 mmol) at 0 °C. The mixture was stirred at the same temperature for 5 min. Then, a solution of the crude mesylate in DMF (2.20 mL) was added dropwise to the reaction mixture. The pale yellow suspension was stirred at rt for 64 h. The mixture was quenched with sat. NH_4Cl and partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0−30% EtOAc in hexane) to give *tert*-butyl 3-((3,4-bis(trifluoromethyl)benzyl)oxy)-azetidine-1-carboxylate (1.07 g, 2.68 mmol, 76%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (9H, s), 3.75 (2H, dd, J = 9.2, 3.5 Hz), 3.98−4.10 (2H, m), 4.34−4.44 (1H, m), 4.63 (2H, s), 7.89 (1H, d, J = 8.1 Hz), 7.99 (1H, s), 8.05 (1H, d, J = 8.1 Hz).

A solution of *tert*-butyl 3-((3,4-bis(trifluoromethyl)benzyl)oxy)-azetidine-1-carboxylate (1.07 g, 2.68 mmol) and *p*-TsOH-H_2O (0.561 g, 2.95 mmol) in EtOAc (13.4 mL) was refluxed for 2 h. After being cooled to rt, the resultant precipitates were collected, washed with EtOAc and dried in vacuo to give **50** (1.17 g, 2.48 mmol, 93%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (3H, s), 3.84−3.99 (2H, m), 4.11−4.26 (2H, m), 4.50 (1H, quin, J = 5.8 Hz), 4.68 (2H, s), 7.11 (2H, d, J = 7.9 Hz), 7.41−7.55 (2H, m), 7.90 (1H, d, J = 8.1 Hz), 8.02 (1H, s), 8.08 (1H, d, J = 8.1 Hz), 8.33-8.96 (2H, m). LCMS (ESI/APCI) m/z 300.1 $[M + H]^+$.

Docking of the Designed Compounds, 2a−c. Docking calculation was performed using the co-crystal structure of **1h** with MAGL (PDB ID: 7L4U) as the template using the Schrodinger suite of software (Release 2016-3). The protein structure was prepared in Protein Preparation Wizard. All water molecules were removed before docking. The compound structure was prepared for docking by LigPrep module and docked using Glide in SP mode. The glide setting was the following, a core constraint by SMARTS patterns, C1CN(C=O)CCC1COc2c(Cl)cc(F)cc2, and the tolerance is 0.50 Å, unchecking canonicalize input conformation and checking enhanced sampling. The docked poses were then minimized using the local optimization feature using MOE2016.0802.

Measurement of MAGL Inhibitory Activity by Mass Spectrometry. His-hMAGL or His-mMAGL was diluted with enzyme reaction buffer (10 mM Tris−HCl, pH 7.5, 1 mM EDTA, 0.025% (w/v) Triton X-100, 0.01% bovine serum albumin) to a concentration of 7.5 or 22.5 ng/mL, respectively. A solution (5 μL) of a test compound dissolved in DMSO and enzyme reaction buffer was added to each well of a 384-well assay plate (Greiner 781280), then 5 μL of 150 μM 2-AG (Tocris Bioscience) was added to each well, and 7.5 ng/mL His-hMAGL or 22.5 ng/mL His-mMAGL solution (5 μL) was added and the mixture and incubated at r.t. for 10 min. Then, 2% formic acid (Wako Pure Chemical Industries, Ltd.) was added by 10 μL to stop the reaction. Furthermore, MeCN (50 μL) containing 3 μM AA-d8 (Cayman Chemical Company) was added, and the mixture was stirred. The amount of AA in the obtained enzyme reaction mixture was calculated by measuring by RapidFire mass spectrometry and correcting by the amount of AA-d8. High-throughput online solid-phase extraction was performed using RapidFire 300 system (Agilent Technologies, Inc.) as described previously.[21] IC_{50} values were calculated by XLfit (IDBS) from the data expressed as inhibition (%) using 4 Parameter Logistic Model. The response of vehicle control was set as the 0% inhibition and the response without enzyme was set as the 100% inhibition. The hMAGL/mMAGL IC_{50} data of **4f** were obtained from five experiments in duplicate, respectively. The hMAGL IC_{50} data of **1b** were obtained from two experiments in duplicate. The hMAGL IC_{50} data of **1**, **1a**, **1c**, **1d**, **1e**, **1f**, **1g**, **1h**, **2a**, **2b**, **2c**, **2d**, **2e**, **2f**, **3a**, **3b**, **3c**, **3d**, **3e**, **3f**, **3g**, **3h**, **3i**, **4a**, **4b**, **4c**, **4d**, **4e**, **4g**, **4h**, **4i**, **4j**, and **4k** were obtained from a single experiment in duplicate.

Measurement of ABHD6 Activity. Human ABHD6 was expressed in Expi293 (Thermo Fisher Scientific). The cells were sonicated, and the lysate was ultracentrifuged at 120000 g for 45 min at 4 °C. The microsome fraction was used as an ABHD6 enzyme. Enzyme and substrate were dissolved in ABHD6 assay buffer [50 mM Tris−HCl, pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, 100 mM NaCl, and 0.1% (w/v) Triton X-100]. Compounds were dissolved in DMSO and then diluted in the ABHD6 assay buffer. Five microliters of compound solution was added to a 384-well clear plate followed by 5 μL of ABHD6 solution (30 μg/mL). Then, 5 μL of substrate solution (3 mM *p*-nitrophenyl acetate) was added and mixed. After the incubation at rt for 30 min, the absorbance at 405 nm was measured using a SpectraMax multilabel plate reader (Molecular Devices). The response of vehicle control was set as the 0% inhibition and the response without enzyme was set as the 100% inhibition.

Surface Plasmon Resonance (SPR)-Based Binding Kinetics. SPR biosensing experiments were performed on a Biacore S200 instrument (GE Healthcare)at 20 °C. The purified human his-MAGL was immobilized on a CM5 sensor chip in an HBS-EP buffer [10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% (v/v) Surfactant P20] containing 1 mM DTT by the standard amine-coupling procedure according to the manufacturer's instructions. One μM human MAGL diluted with 10 mM acetate buffer (pH 5.5) was contacted the surface of the activated chip for 5 min. The immobilization level with approximately 2000 RU was captured onto the sensor chip. The binding kinetics and affinity

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

assay was performed in HBS-EP buffer containing 0.2 mM TCEP and 0.5% DMSO. Data processing and analysis were performed using the Biacore S200 evaluation software (GE healthcare). Sensorgrams were double-referenced prior to global fitting analysis. The curve data were fitted to a 1:1 binding model for the determination of the binding rate constants of association and dissociation (k_{on} and k_{off}). Dissociation constant (K_D) was calculated using the following equation: $K_D = k_{off}/k_{on}$. Dissociative half-lives $t_{1/2}$ were calculated from the dissociation rate constants k_{off} ($t_{1/2} = \ln 2/k_{off}$).

log $D_{pH\ 7.4}$ (log D). log D, which is a partition coefficient between 1-octanol and aqueous buffer pH 7.4, of the compounds was measured by the chromatographic method.[27] log D values of compounds were calculated using a calibration curve based on the retention time of standards. The retention time of the compounds was determined by a UHPLC system of Prominence UFLC with a photodiode array detector (Shimadzu Corp., Kyoto, Japan). A YMC-PackPro C18 packaged column (3 μm, 2.0 mm × 75 mm; YMC Co., Ltd., Kyoto, Japan) was used at 40 °C with an isocratic method. The mobile phase was adjusted methanol/pH 6.5 Britton-Robinson buffer (5:2, v/v) with phosphoric acid to pH 7.4 and a flow rate was 0.2 mL/min.

Aqueous Solubility. Aqueous solubility was measured by the shake flask method.[34] Sample was weighed into Thomson filter vial (Chrom Tech, Inc., Minnesota) containing a 0.45 μm polyvinylidene difluoride filter membrane. A total of 400 μL of second dissolution fluid in the Japanese Pharmacopoeia (JP2, pH 6.8) was added to the vial. The vial was incubated at 37 °C with shaking at 500 rpm for 18 h and filtrated by compressing. Aqueous solubility was determined by a UHPLC system of Prominence UFLC (Shimadzu Corp., Kyoto, Japan) with a photodiode array detector. A YMC-UltraHT Pro C18 packaged column (2 μm, 2.0 mm × 30 mm; YMC Co., Ltd., Kyoto, Japan) was used at 40 °C with the gradient programs. The mobile phase was distilled water/50 mM ammonium acetate buffer/MeCN (8:1:1, v/v/v) and 50 mM ammonium acetate buffer/MeCN (1:9, v/v) and a flow rate was 0.7 mL/min. The starting mobile phase consisted of 95% A and 5% B for 0.1 min, changed gradually to 5% A 210 and 95% B from 0.1 to 1.6 min, and then returned to the initial condition after 1.3 min and was reequilibrated for an additional 0.6 min.

Transcellular Transport Study Using Transporter-Expression System. Human MDR1-expressing LLC-PK1 cells were cultured, and the transcellular transport study was performed with minor modifications to the method reported previously.[37] In brief, the cells were grown in Transwell 96-well permeable support (pore size 0.4 μm, 0.143 cm^2 surface area) with polycarbonate membrane (Corning Life Sciences, Lowell, MA). The cells were preincubated with M199 at 37 °C. Subsequently, transcellular transport was initiated by the addition of M199 either to apical compartments (75 μL) or to basolateral compartments (250 μL) containing 1 μM test compounds. The assay was terminated by the removal of each assay plate after 1 h. Aliquots (25 μL) from the opposite compartments were mixed with acetonitrile, and then centrifuged. The compound concentrations in the supernatant were measured by LC/MS/MS. The apparent permeability (P_{app}) of test compounds in the receiver wells was determined and the efflux ratio (ER, MDR BA/AB) for the MDR1 membrane permeability test was calculated using the following equation

$$\mathrm{ER(MDR\ BA/AB)} = P_{app,BtoA}/P_{app,AtoB}$$

where $P_{app,AtoB}$ and $P_{app,BtoA}$ represent the apparent permeability in the apical-to-basal direction and the basal-to-apical direction, respectively.

Protocol for hERG Electrophysiological Assay Using IonWorks (Autopatch Clamp). The recombinant CHO-K1 cell line expressing the human ether-a-go-go-related gene (CY3038) was purchased from Millipore Corp. and cultured in large batches, frozen, and stored at a density of 1.5 × 10^7 cells per vial to make frozen cells ready to use immediately after thawing. For use, the frozen cells were thawed in Ham's F12 supplemented with 10% fetal bovine serum and 500 μg/mL G418, centrifuged for 3 min at 900 rpm, washed with Dulbecco's phosphate-buffered saline containing $MgCl_2$ and $CaCl_2$ (PBS+), and resuspended in 3 mL of PBS+. The hERG inhibition assay was performed on the IonWorks Quattro (Molecular Devices) system in population patch clamp (PPC) mode. The extracellular solution was PBS with calcium and magnesium (catalog number 14040, Invitrogen). The intracellular solution contained 140 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 20 mM HEPES, pH 7.3, with KOH. After perforation using 100 μg/mL amphotericin B (Sigma-Aldrich), hERG current was measured under the potential-clamp protocol (holding potential −80 mV, the first voltage 40 mV: 2 s, the second voltage −50 mV, 2 s). The peak tail current before addition of the compounds was measured as the pre hERG current. Test compounds were incubated on the cells for a period of 5 min. The peak tail current after addition of the compounds was measured as the post-hERG current. % hERG inhibition was calculated (n = 3 or 4) according to the following equation.

$$\%\mathrm{hERG\ inhibition} = 100 - (\mathrm{post\ hERG\ current}/\mathrm{pre\ hERG\ current}) \times 100$$

Protocol for hERG Electrophysiological Assay Using Manual Patch Clamp. Experiments were performed using HEK 293 cells stably transfected with hERG at room temperature. Whole cell voltage clamp recordings of hERG currents were made using an Axopatch 200B amplifier. Membrane currents were low pass filtered at 1 kHz and sampled at 2.5 kHz. Borosilicate glass pipettes (Harvard Confidential 37 Apparatus, Kent, U.K.) were pulled and fire polished to get final resistances of 2−3 MΩ. Series resistances were less than 6 MΩ and were compensated by 60−85%. The pipette solution contained, KCl 130 mmol/L, NaCl 7 mmol/L, $MgCl_2$ 1 mmol/L, ATP−2Na 5 mmol/L, HEPES 5 mmol/L, EGTA 5 mmol/L, pH 7.2. Cells were perfused with extracellular Tyrode's solution containing, NaCl 137 mmol/L, KCl 4 mmol/L, $MgCl_2$ 1 mmol/L, $CaCl_2$ 1.8 mmol/L, glucose 11 mmol/L, HEPES 10 mmol/L, pH 7.4. The standard voltage protocol to measure the effects of compounds on hERG current amplitudes was to hold the membrane potential at −75 mV and apply 0.5 s depolarizations to 10 mV. Tail currents were evoked with 0.5 s pulses to −40 mV. The protocol was repeated every 5 s, allowing complete current deactivation between test pulses. Experiments were performed at room temperature. The compound was dissolved in DMSO (10 μmol/L) and the solution was added to an external solution to make final compound solution at the concentration of 10 μmol/mL. The peak tail current after addition of the compounds was measured as the post-hERG current. % hERG inhibition was calculated (n = 2) according to the following equation.

$$\%\mathrm{hERG\ inhibition} = 100 - (\mathrm{post\ hERG\ current}/\mathrm{pre\ hERG\ current}) \times 100$$

Ethics Statement. The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited.

Animals. Seven-week-old male C57BL/6J Jcl mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). After acclimation for a week in our laboratory, eight-week-old mice were used for experiments. The animals were housed in a light-controlled room (12-h light/dark cycle with lights on from 7:00 AM). Food and water were provided ad libitum.

Drug Administration. Compound **4f** was suspended in 0.5% (w/v) methylcellulose in distilled water; oral administration (p.o.) was conducted at a volume of 10 mL/kg in mice. A solution of 0.5% methylcellulose was administered as the vehicle control.

In Vivo PK Study of 4f in Mice. These mice were sacrificed 60 min after administration of **4f**, and the brain hemispheres and the plasma were isolated (n = 4 in each group). The brains were rapidly removed and rinsed in ice-cold saline. The hemispheres were then

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

immediately isolated and frozen on dry ice. The isolated samples were stored at −80 °C until use. The frozen samples were homogenized in saline at 4 mL/g tissue except plasma. The plasma and brain concentrations of compounds were measured by high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Measurement of Arachidonic Acid (AA) and 2-Arachidonoylglycerol (2-AG) in Brain. These mice were sacrificed 60 min after administration of **4f**, and the brain hemispheres were isolated (n = 4 in each group). The brains were rapidly removed and rinsed in ice-cold saline. The hemispheres were then immediately dissected and frozen on dry ice. The samples were homogenized with a shake master (Bio Medical Science, Japan), and added 400 uL of isopropanol to 100 mg of tissue weight and mixed well. The suspension (50 uL) was transferred to another tube, and added an equal volume of isopropanol, mixed well, and centrifuged at 15000 rpm for 5 min. The supernatant (5 uL) was transferred to a new tube. The samples were dried up for 10 min under nitrogen spray. The samples were diluted with 100 uL of diluent containing isopropanol. The supernatant (1 uL) was analyzed by LC/MS/MS. The value was calculated from a calibration curve using isopropanol as a matrix. Analysis with LC/MS/MS was outsourced to LSI Medience (Tokyo, Japan).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00432.

Molecular formula strings (CSV)

Analytical HPLC traces for all of the tested compounds, ^{1}H NMR and ^{13}C NMR charts of compound **4f**, co-crystal structure of compound **4f** with MAGL, and X-ray data collection and refinement statistics for the crystal structures of MAGL in complex with compounds **1**, **1h**, **2d**, and **4f** (PDF)

Accession Codes

The coordinates of the crystal structures of MAGL in complex with compounds **1** (7L4T), **1h** (7L4U), **2d** (7L4W), and **4f** (7L50) have been deposited in the Protein Data Bank. The authors will release the atomic coordinates and experimental data upon article publication.

■ AUTHOR INFORMATION

Corresponding Author

Shuhei Ikeda − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan;* orcid.org/0000-0002-0121-2983; Phone: +81 466 32 1198; Email: shuhei.ikeda@takeda.com; Fax: +81 466 29 4450

Authors

Hideyuki Sugiyama − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Hidekazu Tokuhara − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan;* Present Address: Axcelead Drug Discovery Partners, Inc., 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan

Masataka Murakami − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Minoru Nakamura − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Yuya Oguro − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Jumpei Aida − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan;* orcid.org/0000-0002-7546-1915

Nao Morishita − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Satoshi Sogabe − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan;* Present Address: Axcelead Drug Discovery Partners, Inc., 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan; orcid.org/0000-0003-2393-9582

Douglas R. Dougan − *Structural Biology and Biophysics, Takeda California, Inc., San Diego, California 92121, United States*

Sean C. Gay − *Structural Biology and Biophysics, Takeda California, Inc., San Diego, California 92121, United States;* Present Address: Art Robbins Instruments, 1293, Mountain View Alviso Rd, Suite D, Sunnyvale, California 94089, United States.

Ling Qin − *Structural Biology and Biophysics, Takeda California, Inc., San Diego, California 92121, United States*

Naoto Arimura − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Yasuko Takahashi − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Masako Sasaki − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Yusuke Kamada − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan;* Present Address: Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1, Murasaki-cho, Takatsuki, Osaka 569-1125, Japan.; orcid.org/0000-0003-3701-2075

Kazunobu Aoyama − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan;* Present Address: Axcelead Drug Discovery Partners, Inc., 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan

Kouya Kimoto − *Pharmaceutical Sciences, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Makoto Kamata − *Research, Takeda Pharmaceutical Co., Ltd., Fujisawa, Kanagawa 251-8555, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c00432

Author Contributions

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

The authors acknowledge Dr. Tomohiro Kaku for fruitful discussions on ligand design, Dr. Tatsuki Koike for valuable suggestions about research plans, and Dr. Takanobu Kuroita for substantial supports to progress this project. We are also grateful to Dr. Robert Skene for depositing the coordinates of the co-crystal structures.

■ ABBREVIATIONS USED

AA, arachidonic acid; ABHD6, α,β-hydrolase domain 6; ADDP, 1,1′-(azodicarbonyl)dipiperidine; 2-AG, 2-arachido-

noylglycerol; Anandamide, *N*-arachidonoylethanolamine; BBB, blood−brain barrier; BSA, bovine serum albumin; CNS, central nervous system; DBU, 1,8-diazabicyclo[5.4.0]-undec-7-ene; DIPEA, *N,N*-diisopropylethylamine; DME, 1,2-dimethoxyethane; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EC, endocannabinoid; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Et_3N, triethylamine; EtOAc, ethyl acetate; EtOH, ethanol; FAAH, fatty acid amide hydrolase; hERG, human ether-a-go-go-related gene; HOBt, 1-hydroxybenzotriazole; IPE, diisopropyl ether; k_{off}, dissociation rate constant; LLE, lipophilic ligand efficiency; MAGL, monoacylglycerol lipase; MDR1, multidrug resistance protein 1; MeCN, acetonitrile; MeOH, methanol; SBDD, structure-based drug discovery; SPR, surface plasmon resonance; TFAA, trifluoroacetic anhydride; THF, tetrahydrofuran; UHP, urea hydrogen peroxide

REFERENCES

(1) Kano, M.; Ohno-Shosaku, T.; Hashimotodani, Y.; Uchigashima, M.; Watanabe, M. Endocannabinoid-mediated control of synaptic transmission. *Physiol. Rev.* **2009**, *89*, 309−380.

(2) Sugiura, T.; Kondos, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89−97.

(3) Sugiura, T.; Kodaka, T.; Nakane, S.; Miyashita, T.; Kondo, S.; Suhara, Y.; Takayama, H.; Waku, K.; Seki, C.; Baba, N.; Ishima, Y. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. *J. Biol. Chem.* **1999**, *274*, 2794−2801.

(4) Sugiura, T.; Kishimoto, S.; Oka, S.; Gokoh, M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog. Lipid. Res.* **2006**, *45*, 405−446.

(5) Blankman, J. L.; Simon, G. M.; Cravatt, B. F. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* **2007**, *14*, 1347−1356.

(6) (a) Savinainen, J. R.; Saario, S. M.; Laitinen, J. T. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. *Acta Physiol.* **2012**, *204*, 267−276. (b) Bachovchin, D. A.; Cravatt, B. F. The pharmacological landscape and therapeutic potential of serine hydrolases. *Nat. Rev. Drug Discovery* **2012**, *11*, 52−68.

(7) Karlsson, M.; Contreras, J. A.; Hellman, U.; Tornqvist, H.; Holm, C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. *J. Biol. Chem.* **1997**, *272*, 27218−27223.

(8) Dinh, T. P.; Freund, T. F.; Piomelli, D. A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem. Phys. Lipids* **2002**, *121*, 149−158.

(9) Long, J. Z.; Nomura, D. K.; Cravatt, B. F. Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. *Chem. Biol.* **2009**, *16*, 744−753.

(10) Nomura, D. K.; Morrison, B. E.; Blankman, J. L.; Long, J. Z.; Kinsey, S. G.; Marcondes, M. C.; Ward, A. M.; Hahn, Y. K.; Lichtman, A. H.; Conti, B.; Cravatte, B. F. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science* **2011**, *334*, 809−813.

(11) Tuo, W.; Leleu-Chavain, N.; Spencer, J.; Sansook, S.; Millet, R.; Chavatte, P. Therapeutic potential of fatty acid amide hydrolase, monoacylglycerol lipase, and *N*-acylethanolamine acid amidase inhibitors. *J. Med. Chem.* **2017**, *60*, 4−46.

(12) (a) Bononi, G.; Poli, G.; Rizzolio, F.; Tuccinardi, T.; Macchia, M.; Minutolo, F.; Granchi, C. An updated patent review of monoacylglycerol lipase (MAGL) inhibitors (2018-present). *Expert Opin. Ther. Pat.* **2021**, *31*, 153−168. (b) Granchi, C.; Caligiuri, I.; Minutolo, F.; Rizzolio, F.; Tuccinardi, T. A patent review of monoacylglycerol lipase (MAGL) inhibitors (2013-2017). *Expert Opin. Ther. Pat.* **2017**, *27*, 1341−1351.

(13) Kohnz, R. A.; Nomura, D. K. Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids. *Chem. Soc. Rev.* **2014**, *43*, 6859−6869.

(14) Blankman, J. L.; Cravatt, B. F. Chemical probes of endocannabinoid metabolism. *Pharmacol. Rev.* **2013**, *65*, 849−871.

(15) Grabner, G. F.; Zimmermann, R.; Schicho, R.; Taschler, U. Monoglyceride lipase as a drug target: at the crossroads of arachidonic acid metabolism and endocannabinoid signaling. *Pharmacol. Ther.* **2017**, *175*, 35−46.

(16) Sugaya, Y.; Yamazaki, M.; Uchigashima, M.; Kobayashi, K.; Watanabe, M.; Sakimura, K.; Kano, M. Crucial roles of the endocannabinoid 2-arachidonoylglycerol in the suppression of epileptic seizures. *Cell Rep.* **2016**, *16*, 1405−1415.

(17) Cisar, J. S.; Weber, O. D.; Clapper, J. R.; Blankman, J. L.; Henry, C. L.; Simon, G. M.; Alexander, J. P.; Jones, T. K.; Ezekowitz, R. A. B.; O'Neill, G. P.; Grice, C. A. Identification of ABX-1431, a selective inhibitor of monoacylglycerol lipase and clinical candidate for treatment of neurological disorders. *J. Med. Chem.* **2018**, *61*, 9062−9084.

(18) McAllister, L. A.; Butler, C. R.; Mente, S.; O'Neil, S. V.; Fonseca, K. R.; Piro, J. R.; Cianfrogna, J. A.; Foley, T. L.; Gilbert, A. M.; Harris, A. R.; Helal, C. J.; Johnson, D. S.; Montgomery, J. I.; Nason, D. M.; Noell, S.; Pandit, J.; Rogers, B. N.; Samad, T. A.; Shaffer, C. L.; da Silva, R. G.; Uccello, D. P.; Webb, D.; Brodney, M. A. Discovery of trifluoromethyl glycol carbamates as potent and selective covalent monoacylglycerol lipase (magl) inhibitors for treatment of neuroinflammation. *J. Med. Chem.* **2018**, *61*, 3008−3026.

(19) Butler, C. R.; Beck, E. M.; Haris, A.; Huang, Z.; McAllister, L. A.; am Ende, C. W.; Fennel, K.; Foley, T. L.; Fonseca, K.; Hawrylik, S. J.; Johnson, D. S.; Knafels, J. D.; Mente, S.; Noell, G. S.; Pandit, J.; Phillips, T. B.; Piro, J. R.; Rogers, B. N.; Samad, T. A.; Wang, J.; Wan, S.; Brodney, M. A. Azetidine and piperidine carbamates as efficient, covalent inhibitors of monoacylglycerol lipase. *J. Med. Chem.* **2017**, *60*, 9860−9873.

(20) Schlosburg, J. E.; Blankman, J. L.; Long, J. Z.; Nomura, D. K.; Pan, B.; Kinsey, S. G.; Nguyen, P. T.; Ramesh, D.; Booker, L.; Burston, J. J.; Thomas, E. A.; Selley, D. E.; Sim-Selley, L. J.; Liu, Q. S.; Lichtman, A. H.; Cravatt, B. F. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat. Neurosci.* **2010**, *13*, 1113−1119.

(21) Aida, J.; Fushimi, M.; Kusumoto, T.; Sugiyama, H.; Arimura, N.; Ikeda, S.; Sasaki, M.; Sogabe, S.; Aoyama, K.; Koike, T. Design, synthesis, and evaluation of piperazinyl pyrolidin-2-ones as a novel series of reversible monoacylglycerol lipase inhibitors. *J. Med. Chem.* **2018**, *61*, 9205−9217.

(22) (a) Granchi, C.; Lapillo, M.; Glasmacher, S.; Bononi, G.; Licari, C.; Poli, G.; Boustani, M.; Caligiuri, I.; Rizzolio, F.; Gertsch, J.; Macchia, M.; Minutolo, F.; Tuccinardi, T.; Chicca, A. Optimization of a benzoylpiperidine class identifies a highly potent and selective reversible monoacylglycerol Lipase (MAGL) Inhibitor. *J. Med. Chem.* **2019**, *62*, 1932−1958. (b) Aghazadeh Tabrizi, M.; Baraldi, P. G.; Baraldi, S.; Ruggiero, E.; Stefano, L. D.; Rizzolio, F.; Mannelli, L. D. C.; Ghelardini, C.; Chicca, A.; Lapillo, M.; Gertsch, J.; Manera, C.; Macchia, M.; Martinelli, A.; Granchi, C.; Minutolo, F.; Tuccinardi, T. Discovery of 1,5-diphenylpyrazole-3-carboxamide derivatives as potent, reversible, and selective monoacylglycerol lipase (MAGL) inhibitors. *J. Med. Chem.* **2018**, *61*, 1340−1354. (c) Granchi, C.; Rizzolio, F.; Palazzolo, S.; Carmignani, S.; Macchia, M.; Saccomanni, G.; Manera, C.; Martinelli, A.; Minutolo, F.; Tuccinardi, T. Structural optimization of 4-chlorobenzoylpiperidine derivatives for the development of potent, reversible, and selective monoacylglycerol lipase (MAGL) inhibitors. *J. Med. Chem.* **2016**, *59*, 10299−10314.

(23) Hernández-Torres, G.; Cipriano, M.; Hedén, E.; Björklund, E.; Canales, Á.; Zian, D.; Feliú, A.; Mecha, M.; Guaza, C.; Fowler,

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX

C. J.; Ortega-Gutiérrez, S.; López-Rodríguez, M. L. A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis. *Angew. Chem., Int. Ed.* **2014**, *53*, 13765−13770.
(24) (a) Wyatt, R. M.; Fraser, I.; Welty, N.; Lord, B.; Wennerholm, M.; Sutton, S.; Ameriks, M. K.; Dugovic, C.; Yun, S.; White, A.; Nguyen, L.; Koudriakova, T.; Tian, G.; Suarez, J.; Szewczuk, L.; Bonnette, W.; Ahn, K.; Ghosh, B.; Flores, C. M.; Connolly, P. J.; Zhu, B.; Macielad, M. J.; Brandt, M. R.; Chevalier, K.; Zhang, S.; Lovenberg, T.; Bonaventure, P. Pharmacologic characterization of JNJ-42226314, [1-(4-fluorophenyl)indol-5-yl]-[3-[4-(thiazole-2-carbonyl)piperazin-1-yl]azetidin-1-yl]methanone, a reversible, selective and potent monoacylglycerol lipase inhibitor. *J. Pharmacol. Exp. Ther.* **2020**, *372*, 339−353. (b) Zhu, B.; Connolly, P. J.; Zhang, S.; Chevalier, K. M.; Milligan, C. M.; Flores, C. M.; Macielag, M. J. The discovery of diazetidinyl diamides as potent and reversible inhibitors of monoacylglycerol lipase (MAGL). *Bioorg. Med. Chem. Lett.* **2020**, *30*, No. 127198. (c) Zhu, B.; Connolly, P. J.; Zhang, Y.; McDonnell, M. E.; Bian, H.; Lin, S.; Liu, L.; Zhang, S.; Chevalier, K. M.; Brandt, M. R.; Milligan, C. M.; Flores, C. M.; Macielag, M. J. The discovery of azetidine-piperazine di-amides as potent, selective and reversible monoacylglycerol lipase (MAGL) inhibitors. *Bioorg. Med. Chem. Lett.* **2020**, *30*, No. 127243.
(25) King, A. R.; Dotsey, E. Y.; Lodola, A.; Jung, K. M.; Ghomian, A.; Qiu, Y.; Fu, J.; Mor, M.; Piomelli, D. Discovery of potent and reversible monoacylglycerol lipase inhibitors. *Chem. Biol.* **2009**, *16*, 1045−1052.
(26) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discovery* **2007**, *6*, 881−890.
(27) Yamamoto, K.; Ikeda, Y. Kinetic solubility and lipophilicity evaluation connecting formulation technology strategy perspective. *J. Drug Delivery Sci. Technol.* **2016**, *33*, 13−18.
(28) Bertrand, T.; Augé, F.; Houtmann, J.; Rak, A.; Vallée, F.; Mikol, V.; Berne, P. F.; Michot, N.; Cheuret, D.; Hoornaert, C.; Mathieu, M. Structural basis for human monoglyceride lipase inhibition. *J. Mol. Biol.* **2010**, *396*, 663−673.
(29) Schalk-Hihi, C.; Schubert, C.; Alexander, R.; Bayoumy, S.; Clemente, J. C.; Deckman, I.; DesJarlais, R. L.; Dzordzorme, K. C.; Flores, C. M.; Grasberger, B.; Kranz, J. K.; Lewandowski, F.; Liu, L.; Ma, H.; Maguire, D.; Macielag, M. J.; McDonnell, M. E.; Mezzasalma Haarlander, T.; Miller, R.; Milligan, C.; Reynolds, C.; Kuo, L. C. Crystal structure of a soluble form of human monoglyceride lipase in complex with an inhibitor at 1.35 Å resolution. *Protein Sci.* **2011**, *20*, 670−683.
(30) Bissantz, C.; Kuhn, B.; Stahl, M. A medicinal chemist's guide to molecular interactions. *J. Med. Chem.* **2010**, *53*, 5061−5084.
(31) Giacomini, K. M.; Huang, S-M.; Tweedie, D. J.; Benet, L. Z.; Brouwer, K. L. R.; Chu, X.; Dahlin, A.; Evers, R.; Fischer, V.; Hillgren, K. M.; Hoffmaster, K. A.; Ishikawa, T.; Keppler, D.; Kim, R. B.; Lee, C. A.; Niemi, M.; Polli, J. W.; Sugiyama, Y.; Swaan, P. W.; Ware, J. A.; Wright, S. H.; Yee, S. W.; Zamek-Gliszczynski, M. J.; Zhang, L. Membrane transporters in drug development. *Nat. Rev. Drug Discovery* **2010**, *9*, 215−236.
(32) Rankovic, Z. CNS drug design: balancing physicochemical properties for optimal brain exposure. *J. Med. Chem.* **2015**, *58*, 2584−2608.
(33) Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009**, *52*, 6752−6756.
(34) Nakashima, S.; Yamamoto, K.; Arai, Y.; Ikeda, Y. Impact of physicochemical profiling for rational approach on drug discovery. *Chem. Pharm. Bull.* **2013**, *61*, 1228−1238.
(35) Kono, M.; Matsumoto, T.; Imaeda, T.; Kawamura, T.; Fujimoto, S.; Kosugi, Y.; Odani, T.; Shimizu, Y.; Matsui, H.; Shimojo, M.; Kori, M. Design, synthesis, and biological evaluation of a series of piperazine ureas as fatty acid amide hydrolase inhibitors. *Bioorg. Med. Chem.* **2014**, *22*, 1468−1478.
(36) Brooks, G.; Dabbs, S.; Davies, D. T.; Hennessy, A. J.; Jones, G. E.; Markwell, R. E.; Miles, T. J.; Owston, N. A.; Pearson, N. D.; Peng, T. W. The design of efficient and selective routes to pyridyl analogues of 3-oxo-3,4-dihydro-2*H*-1,4-(benzothiazine or benzoxazine)-6-carbaldehydes. *Tetrahedron. Lett.* **2010**, *51*, 5035−5037.
(37) Takeuchi, T.; Yoshitomi, S.; Higuchi, T.; Ikemoto, K.; Niwa, S.; Ebihara, T.; Katoh, M.; Yokoi, T.; Asahi, S. Establishment and characterization of the transformants stably-expressing MDR1 derived from various animal species in LLC-PK1. *Pharm. Res.* **2006**, *23*, 1460−1472.

https://doi.org/10.1021/acs.jmedchem.1c00432
J. Med. Chem. XXXX, XXX, XXX−XXX