Journal of Medicinal Chemistry

pubs.acs.org/jmc

Article

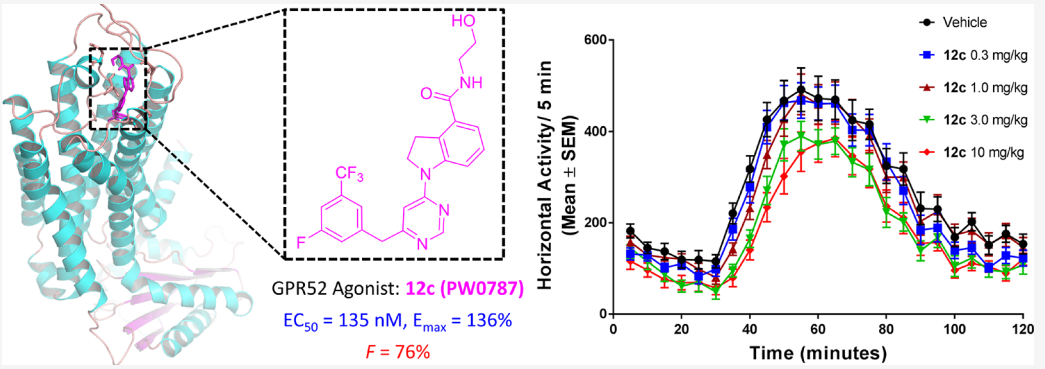
Discovery of Potent and Brain-Penetrant GPR52 Agonist that Suppresses Psychostimulant Behavior

Pingyuan Wang,[§] Daniel E. Felsing,[§] Haiying Chen, Sonja J. Stutz, Ryan E. Murphy, Kathryn A. Cunningham, John A. Allen,*[,∥] and Jia Zhou*[,∥]

Cite This: https://dx.doi.org/10.1021/acs.jmedchem.0c01498

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: The G protein-coupled receptor 52 (GPR52) is an orphan receptor that is selectively expressed in the striatum and regulates various brain functions through activation of cAMP-dependent pathways. GPR52 has been identified as a promising therapeutic target for central nervous system disorders including schizophrenia and substance use disorders. Here, a series of novel GPR52 agonists were designed, synthesized, and evaluated based on compound **4**. Several potent and efficacious GPR52 agonists (**12c**, **23a**, **23d**, **23e**, **23f**, and **23h**) were identified with nanomolar range potency based on a systematic structure−activity relationship exploration. Further studies of **12c** indicate enhanced efficacy, excellent target selectivity, and pharmacokinetic properties including good brain permeability. *In vivo* proof-of-concept investigations revealed that **12c** displayed antipsychotic-like activity by significantly inhibiting amphetamine-induced hyperlocomotor behavior in mice. Collectively, our findings have resulted in an efficacious, brain-penetrant GPR52 agonist as a valuable pharmacological tool for investigating the physiological and therapeutic potential of GPR52 activation.

INTRODUCTION

G-protein-coupled receptors (GPCRs) are a superfamily of seven-transmembrane spanning receptor proteins, with over 800 members. GPCRs play critical roles in cellular communication and regulate a wide range of physiological and pathological processes.[1] To date, GPCRs have accounted for approximately 30% of all U.S. Food and Drug Administration (FDA)-approved drugs for the therapies of numerous human diseases including cancer, central nervous system (CNS) disorders, metabolic disorders, inflammation diseases, infection, immune diseases, and others.[2−5] However, only a fraction of GPCRs are successfully targeted in drug development,[6] and roughly one third of the non-sensory GPCRs remain orphan receptors whose endogenous ligands and physiological functions are largely unknown. Despite limited knowledge of these orphan GPCRs, their crucial roles in physiological signaling pathways and putative druggability make them attractive novel therapeutic targets.[2,7,8]

GPR52 is an understudied brain orphan receptor selectively expressed in the striatum and cortex with the highest level of expression in the nucleus accumbens (NAc).[9,10] The selective expression of GPR52 in the NAc and other striatal regions suggests an important function for this receptor in primary striatal neurons and broader corticostriatal circuitry.[11] Moreover, GPR52 is colocalized exclusively with dopamine D2 receptors in medium spiny neurons (MSNs) in the striatum and has lesser expression in neurons of the medial prefrontal cortex.[10] Notably in transgenic mice, the overexpression of GPR52 significantly decreased methamphetamine-induced locomotion, while GPR52 knockout mice showed an

Received: August 27, 2020

anxiolytic-like phenotype.[10] Studies have indicated that GPR52 couples to $G_{as/olf}$- G proteins to activate adenylyl cyclase and modulate 5′-cyclic adenosine monophosphate (cAMP) signaling and displays high levels of constitutive activity.[12,13] Thus, GPR52 signaling via cAMP could oppose activity of D2 signaling in the striatum while stimulating the D1/*N*-methyl-D-aspartate (NMDA) function in the frontal cortex.[14] Therefore, GPR52 may serve as a promising novel target for psychiatric disorders[10] including schizophrenia[15] and substance use disorders (SUDs).[16]

To date, the natural ligand of GPR52 has not been elucidated, but several surrogate ligands have been reported.[14,16−18] Most recently, Lin et al. found that the orthosteric ligand binding pocket of GPR52 (PDB codes: 6LI1 and 6LI2) was occupied by its extracellular loop 2 (ECL2).[12] They suggested that GPR52 was an unprecedented self-activating receptor and GPR52 ligands could function as allosteric agonists to control GPR52 self-activation.[12] Compound **1** (Figure 1) is the first reported potent GPR52 agonist

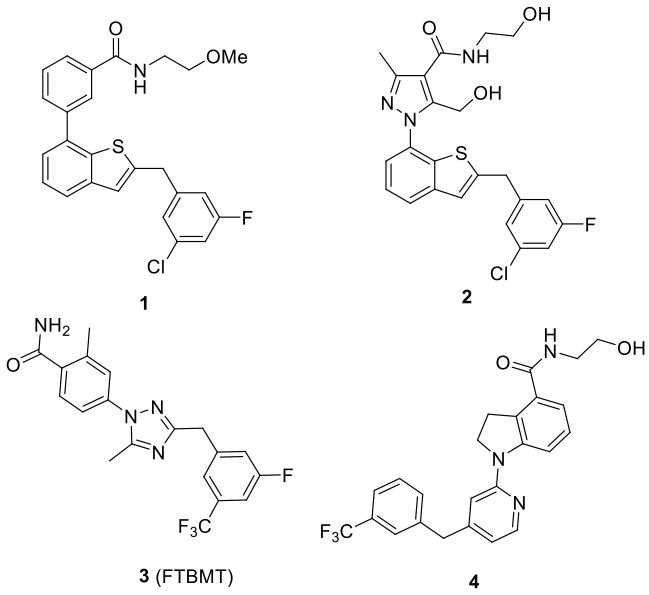


Figure 1. Structures of representative reported GPR52 agonists.

with a half maximal effective concentration (EC_{50}) of about 30 nM, on the basis of their high-throughput screening (HTS) hits.[14] Particularly, compound **1** had excellent bioavailability (F = 73%) and brain penetration (brain/plasma AUC ratio = 0.94).[14] Additionally, compound **1** significantly inhibited rodent methamphetamine-induced hyperactivity at a dose of 3 mg/kg. However, compound **1** has some drawbacks including high lipophilicity/poor aqueous solubility, which limit its application for further use.[18] Further modifications of compound **1** resulted in optimized compound **2** (Figure 1) with slightly improved GPR52 potency (EC_{50} = 21 nM and E_{max} = 103%) and water solubility (21 μg/mL at pH 6.8) and similar activity to inhibit stimulant-induced hyperactivity.[18] Interestingly, the cocrystal structure of GPR52 and compound **2** (PDB code: 6LI0) was recently solved.[12] Compound **2** was found located in a small extracellular pocket of GPR52 surrounded by ECL2, transmembrane helix 1 (TM1), TM2, and TM7. The agonist formed multiple interactions with GPR52 including hydrogen bonds with residues Cys40, Glu191, Ile189, and Asp188 in ECL2, π−π stacking with residue Phe300 of TM7, and hydrophobic contacts with residues Phe117, Thr303, Trp304, and Ile307. The elucidation of this GPR52 ligand-binding pocket is anticipated to facilitate further drug discovery of potent and selective GPR52 modulators. Recently, Tokumaru et al. reported another series of novel GPR52 agonists.[17] Among those GPR52 agonists, compound **3** (Figure 1) was an effective agonist with good potency and efficacy (EC_{50} of 75 nM and E_{max} of 122%).[17] Animal model studies indicated that compound **3** inhibited MK-801-induced hyperactivity and improved memory recognition.[15] Likewise, Arena Pharmaceuticals patented and disclosed a series of 1-heteroaryl-indoline-4-carboxamines as GPR52 agonists, among which, compound **4** displayed potent GPR52 agonist activity.[16] Here, we choose compound **4** as a starting point for our medicinal chemistry efforts due to its amenability to chemical synthesis, optimal physical−chemical properties, and lack of previously known structure−activity relationship (SAR). Taken together, GPR52 is emerging as a promising neurotherapeutic target of interest for the treatment of schizophrenia and SUDs, and the reported agonists as well as the recently disclosed cocrystal structural analysis serve as new starting points to facilitate further drug discovery of novel GPR52 ligands as pharmacological tools and potential drug candidates for preclinical and clinical development.

As part of our ongoing research program in understanding the GPR52-associated pharmacology and identifying novel ligands through HTS campaign and structure-based rational drug design, herein, we report our findings in the chemical optimization and pharmacological evaluation of novel GPR52 activators based on compound **4**[16] as the advanced chemical lead to understand the key interactions of agonists with the receptor and to identify improved ligands. Systematic SAR studies around three pharmacological moieties of compound **4** have been explored. A series of novel 1-(pyrimidin-4-yl)indoline-4-carboxamide analogues have been identified as potent and selective GPR52 agonists with nanomolar range potency and varied levels of improved efficacy. This work culminated in discovery of compound **12c** (**PW0787**) with a new pyrimidine core scaffold that is an orally bioavailable, brain-penetrant, potent, and selective GPR52 agonist. This novel ligand dose-dependently suppresses amphetamine-evoked hyperactivity, suggesting a therapeutic potential for neuropsychiatric diseases.

RESULTS AND DISCUSSION

Design. As described in the Introduction, one of the drawbacks of previously reported GPR52 agonists is the poor aqueous solubility, limiting their use as pharmacological tools or potential drug candidates. The replacement of a CH group with a N atom in aromatic and heteroaromatic rings of the pharmacophore could improve its physicochemical properties for better *in vivo* pharmacokinetic (PK) properties.[19−21] Additionally, compared to the CH group, the N atom has an extra unshared electron pair, which could form hydrogen bonds to potentially improve binding affinity. On this basis, we hypothesized that an extra N atom in the core heteroaromatic ring B of advanced chemical lead **4** could improve its potency as well as physicochemical properties. Therefore, we first altered moiety B (Figure 2, highlight in magenta) by insertion of a nitrogen atom in the pyridine ring of compound **4** as the starting point to systematically pursue SAR studies. Next, we substituted various moieties onto the aromatic ring of moiety C (Figure 2, highlight in cyan). Then, the methylene linker

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

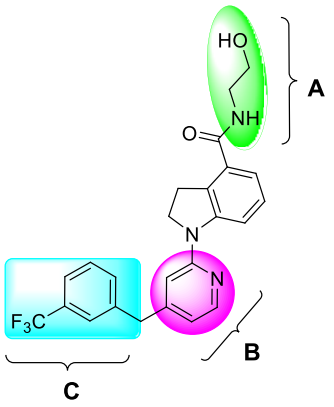


Figure 2. Proposed structural modifications of three substructures (A, B, and C) based on advanced chemical lead **4** for SAR exploration to understand the key interactions of agonists with the receptor GPR52 and to identify newer ligands.

between moieties B and C was also investigated. Finally, we investigated substituents on moiety A (Figure 2, highlight in green) of compound **4** by replacement of aminoethanol with various side chains to understand the critical ligand−receptor interactions.

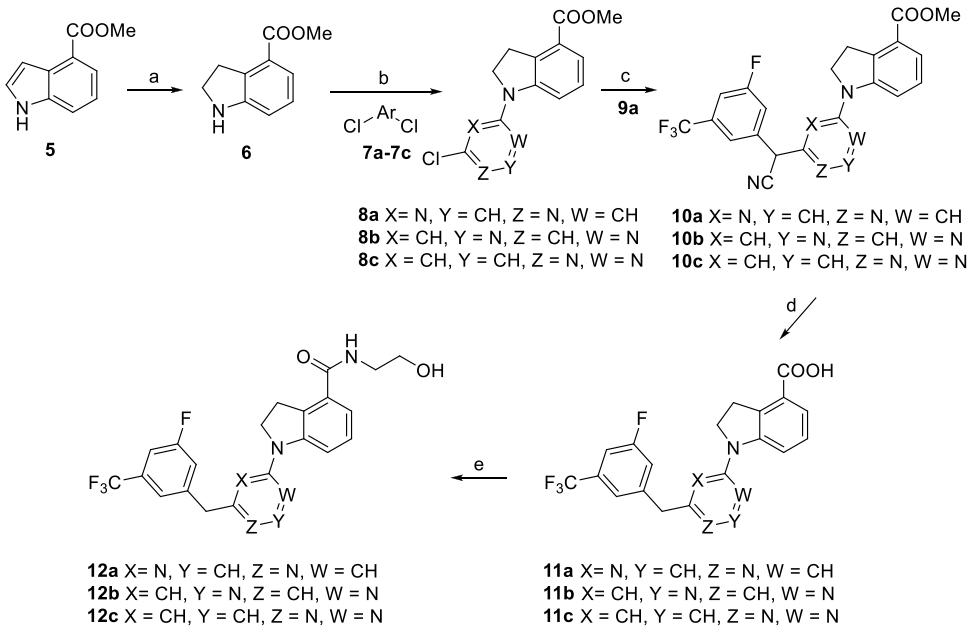
Chemistry. The synthetic procedures of these newly synthesized GPR52 agonists are depicted in Schemes 1−7. As outlined in Scheme 1, intermediate **6** was prepared by reduction of starting material **5** with triethylsilane and trifluoroacetic acid.[22] Intermediates **8a−c** were generated by reaction of compound **6** with commercially available compounds **7a−c** in the presence of Et_3N in yields of 63−67%.[23] Intermediates **10a−c** were produced via nucleophilic substitution of compounds **8a−c** with commercially available 2-(3-fluoro-5-(trifluoromethyl)phenyl)acetonitrile (**9a**).[24] Refluxing of intermediates **10a−c** in the HCl/AcOH/H_2O mixed solvent system led to key intermediates **11a−c** in excellent yields.[24] Coupling of intermediates **11a−c** with aminoethanol produced compounds **12a−c** and resulted in good yields (73−83%).

As outlined in Scheme 2, intermediate **13** was obtained by reaction of compound **9a** with **7a** in the presence of NaH. Refluxing of intermediate **13** in the HCl/AcOH/H_2O solvent system followed by chlorination with phosphorus oxychloride produced compound **14**. Compound **15** was prepared *via* palladium-catalyzed C−N coupling reaction of **14** with **6** in a yield of 56%. Hydrolysis of intermediate **15** generated key intermediate acid **16**. Further, condensation of compound **16** with aminoethanol afforded compound **12d** following the same procedure as that of preparing compound **12a**.

As outlined in Scheme 3, with starting material **9a** and compounds **7d**[25] and **7e**,[25] intermediates **17a** and **17b** were obtained following the similar synthetic procedure to that of preparing compound **13**, respectively. Removing the cyano and the tetrahydro-2*H*-pyran protection groups of **17a** and **17b** at the same time led to compounds **18a** and **18b** in the HCl/AcOH/H_2O solvent system. Compounds **18a** and **18b** were converted into compounds **19a** and **19b** by a standard chlorination reaction. Key intermediates **20a** and **20b** were synthesized by C−N coupling reaction of intermediates **19a** and **19b** with compound **6**, respectively, under the palladium-catalyzed conditions. Acids **21a** and **21b** were prepared by hydrolysis of intermediates **20a** and **20b**. Compounds **12e** and **12f** were synthesized following the similar procedure to that of preparing **12a** by coupling **21a** and **21b** with aminoethanol, respectively.

Compounds **23a−k** were synthesized following the same procedures for preparing **12a** from various commercially available substituted 2-phenylacetonitriles (**9b−l**) (Scheme 4). As outlined in Scheme 5, intermediate **24a** was obtained by reaction of compound **9a** with compound **7a** in the presence of

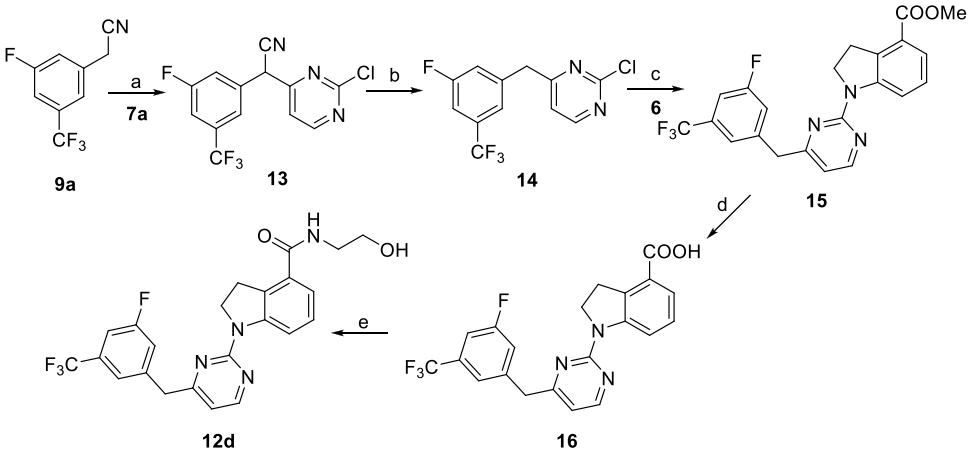
Scheme 1. Synthetic Routes of Compounds 12a−c[a]



[a]Reagents and conditions: (a) CF_3COOH, Et_3SiH, CH_2Cl_2, overnight, and 89%. (b) Et_3N, EtOH, rt., overnight, and 63−67%. (c) 2-(3-Fluoro-5-(trifluoromethyl)phenyl)acetonitrile (**9a**), NaH, DMF, 0 °C to rt., 2 h, and 53−66%. (d) Con. HCl/AcOH/H_2O, reflux, overnight, and 91−93%. (e) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, and 73−83%.

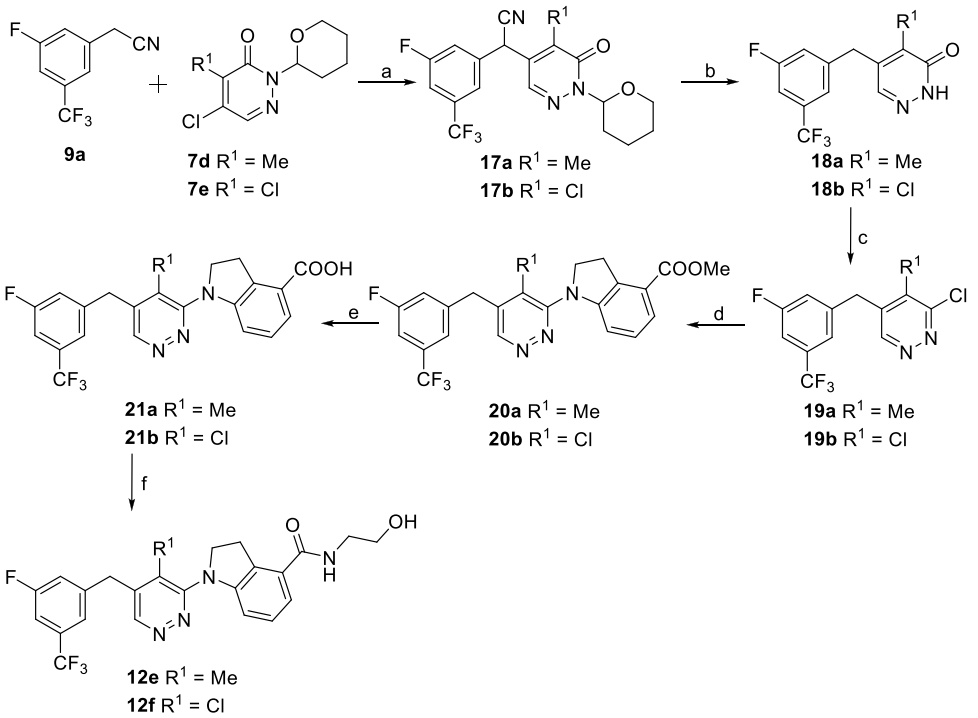
https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthetic Routes of Compound 12d[a]



[a]Reagents and conditions: (a) NaH, DMF, 0 °C to rt., 2 h, and 73%. (b) (1) Con. HCl/AcOH/H_2O, reflux, and overnight; (2) $POCl_3$, reflux, 3 h, and 51% for two steps. (c) **6**, $Pd(OAc)_2$, XantPhos, Cs_2CO_3, 1,4-dioxane, 100 °C, overnight, and 56%. (d) (1) 2 N NaOH, MeOH, reflux, and 1 h; (2) 2 N HCl, rt., and 95%. (e) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, and 83%.

Scheme 3. Synthetic Routes of Compounds 12e and 12f[a]



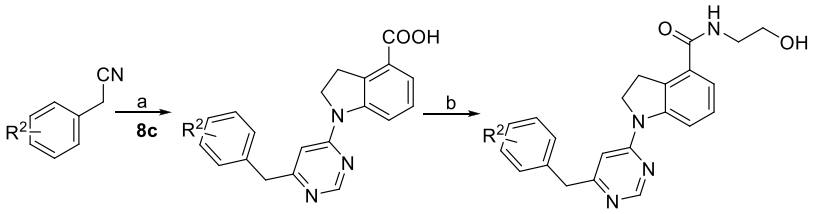
[a]Reagents and conditions: (a) NaH, DMF, 0 °C to rt., 2 h, 36% for **17a**, and 63% for **17b**. (b) HCl, AcOH, H_2O, 120 °C, overnight, 78% for **18a**, and 83% for **18b**. (c) $POCl_3$, 120 °C, 12 h, 83% for **19a**, and 80% for **19b**. (d) **6**, $Pd(OAc)_2$, XantPhos, Cs_2CO_3, 1,4-dioxnae, 100 °C, overnight, 64% for **20a**, and 60% for **20b**. (e) (i) 2 N NaOH, MeOH, reflux, and 1 h; (ii) 2 N HCl, rt., 92% for **21a**, and 90% for **21b**. (f) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, 79% for **12e**, and 59% for **12f**.

NaH followed by oxidation of the intermediate and then coupling with compound **6**. Compounds **24b** and **24c** were synthesized *via* reaction of compounds **9a** and **9b** with compound **8c**, respectively, following similar processes to the synthesis of compound **24a**. Hydrolysis of esters **24a–c** followed by condensation with aminoethanol provided compounds **25a–c** in yields of 63–77% over two steps. Treatment of **25a–c** with $NaBH_4$ as the reducing agent afforded compounds **25d–f** accordingly. Ester intermediates **24a–c** were converted into intermediates **26a–c** by a reduction reaction and then a hydrolysis reaction. Fluoridation of acid intermediates **26a–c** by using diethylaminosulfur trifluoride followed by hydrolysis reactions led to acid compounds **27a–c**. Compounds **25g–i** were produced following the same procedure as that of preparing **12a** from compounds **27a–c**.

As depicted in Scheme 6, compounds **29a–c** were produced *via* coupling reaction of **8c** with corresponding commercially available compounds **28a–c** in yields of 63–71%. Subsequently, intermediates **29a–c** were converted to correspond-

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX–XXX

Scheme 4. Synthetic Routes of Compounds 23a−k[a]



9b R^2 = 3-CF_3	**22a** R^2 = 3-CF_3	**23a** R^2 = 3-CF_3
9c R^2 = 2-CF_3	**22b** R^2 = 2-CF_3	**23b** R^2 = 2-CF_3
9d R^2 = 4-CF_3	**22c** R^2 = 4-CF_3	**23c** R^2 = 4-CF_3
9e R^2 = 3-Me	**22d** R^2 = 3-Me	**23d** R^2 = 3-Me
9f R^2 = 3-F	**22e** R^2 = 3-F	**23e** R^2 = 3-F
9g R^2 = 3,5-di-Me	**22f** R^2 = 3,5-di-Me	**23f** R^2 = 3,5-di-Me
9h R^2 = 3,5-di-F	**22g** R^2 = 3,5-di-F	**23g** R^2 = 3,5-di-F
9i R^2 = 3-OCF_3	**22h** R^2 = 3-OCF_3	**23h** R^2 = 3-OCF_3
9j R^2 = 3-OMe	**22i** R^2 = 3-OMe	**23i** R^2 = 3-OMe
9k R^2 = 3,5-di-OMe	**22j** R^2 = 3,5-di-OMe	**23j** R^2 = 3,5-di-OMe
9l R^2 = H	**22k** R^2 = H	**23k** R^2 = H

[a]Reagents and conditions: (a) (1) NaH, DMF, 0 °C to rt., and 2 h; (2) Con. HCl/AcOH/H_2O, reflux, overnight, and 54−90% for two steps. (b) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, and 29−83%.

ing final compounds **25j−l** by acidolysis and coupling with aminoethanol, following the similar procedures to that of preparing **25a**. Compounds **30a−g** were prepared by coupling **11c** with corresponding aminoethanol derivatives following the similar procedure to that of preparing **12a**, as outlined in Scheme 7.

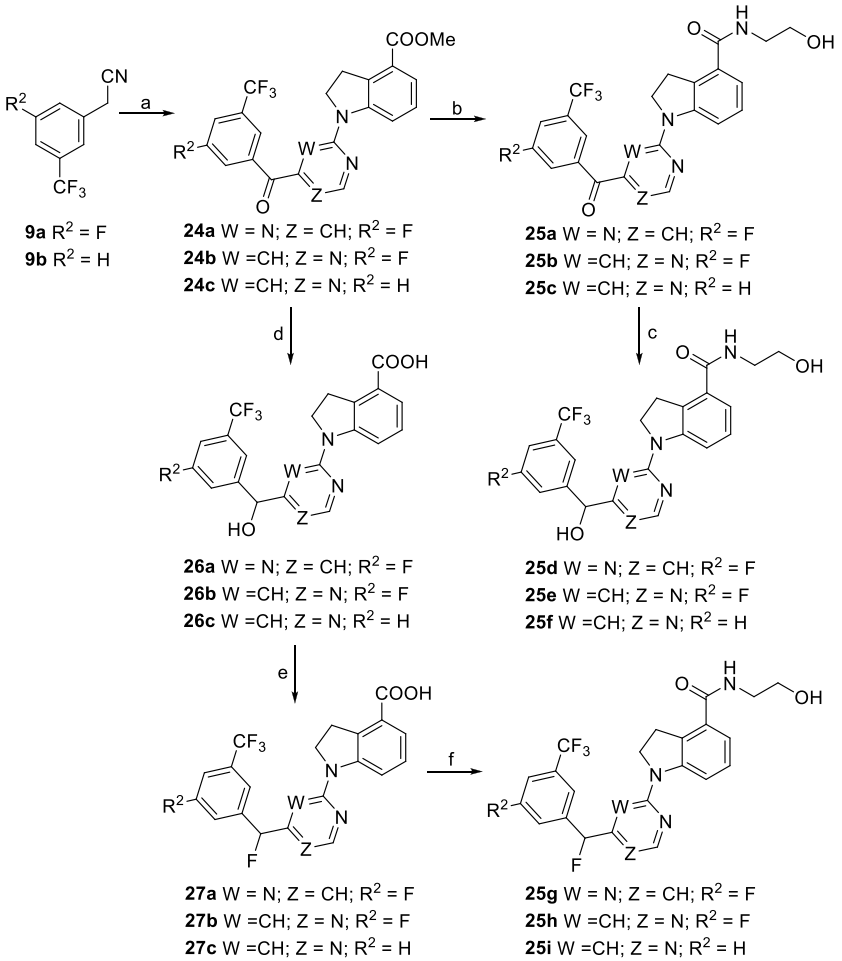
***In Vitro* Evaluation of GPR52 Activation.** Newly synthesized compounds were evaluated in a twelve-point concentration response for GPR52 agonist activity using the Glosensor cAMP assay in HEK293 cells transiently expressing human GPR52. Transient expression of GPR52 resulted in a dramatic increase in basal cAMP levels (>100-fold over an empty vector, Supporting Information, Figure S1), indicating that GPR52 displays high constitutive activity for the cAMP pathway. As summarized in Tables 1−4, the EC_{50} (nM) indicates the potency of these novel GPR52 agonists, while the efficacy (E_{max}) indicates the maximal activity to increase cAMP. Previously reported GPR52 agonist compound **4** was used as the reference compound to highlight SAR comparisons.[16] E_{max} (%) was evaluated with compound **4** as 100% and 0.5% DMSO (vehicle control) as 0%. Compound **4** exhibited very good potency and robust efficacy in the cAMP assay (EC_{50} of 119 nM and E_{max} of ∼3-fold over basal values, Supporting Information, Figure S1). All synthesized ligands were screened in the assay in a full concentration response (0.1 nM to 30 μM). Some data points, at higher concentrations, were excluded from pharmacological analysis due to limited aqueous solubility (as shown in Supporting Information, Figure S1).

As summarized in Table 1, to begin a systematic SAR study of the scaffold of compound **4**,[16] we first investigated the core ring B (Figure 2, highlight in magenta) by insertion of an additional N atom in the pyridine ring of compound **4**. Replacement of the pyridine ring by a pyrimidine ring with two N atoms at positions 2 and 4, leading to compound **12a**, resulted in ∼3-fold loss of potency with an EC_{50} value of 373 nM relative to compound **4**. However, compared to compound **4**, the efficacy of compound **12a** increased with an E_{max} of 144%. In addition, **12a** has a more favorable ClogP relative to that of **4** (3.64 *vs* 4.11), suggesting that **12a** may have a better PK profile. In the further SAR studies, the pyridine ring of compound **4** was replaced with a pyridazine ring to obtain compound **12b** (EC_{50} = 158 nM and E_{max} = 158%), which exhibited comparable potency to that of **4**, with improved efficacy. Next, modification of the pyridine ring of compound **4** to a pyrimidine ring with the two N atoms fixed at positions 4 and 6 led to compound **12c** (Table 1 and Figure 3A). Compound **12c** showed equal potency as that of lead **4** (135 nM *vs* 119 nM) but interestingly resulted in a superior efficacy (E_{max} of 136%), which was the best compound among this series. However, when the two N atoms were moved to positions 2 and 6 to obtain **12d**, the potency decreased slightly (EC_{50} = 186 nM) compared to **12c**. To explore the effect of adding a substituent on potency, we introduced a methyl or a chlorine at the 2-position on compound **12b**, leading to compounds **12e** and **12f**, respectively. However, both compounds showed substantial loss of potency (>4-fold decreased EC_{50}), indicating that adding a substituent on core moiety B was not favorable.

Having identified an optimal heterocycle in moiety B that maintains potency while increasing efficacy, we next focused on probing the role of the substituents on the benzene ring of moiety C (Figure 2, highlight in cyan), with results summarized in Table 2. To evaluate the importance of the F substituent, we removed the F from compound **12c**, leading to compound **23a**, resulting in a slight increase in potency (EC_{50} of 101 nM) and minor decrease in efficacy relative to compound **12c** (127% *vs* 136%) (Table 2). Compounds **23b** and **23c** were then designed and synthesized to explore whether the CF_3 position had an influence on agonist potency. CF_3 at the *ortho*-position (**23b**) resulted in a dramatic loss of potency (>7-fold loss) compared with CF_3 at the *meta*-position (**23a**). Remarkably, when the CF_3 moiety was moved to the *para*-position (**23c**), a similar potency and efficacy was observed to that of **23a** (109 nM *vs* 101 nM for EC_{50} and 136% *vs* 127% for E_{max} (Table 2 and Figure 3B)). From these results, we can conclude that the substituent position on the benzene ring of moiety C has an important role in compound potency and their preferred order is the *meta* > *para* > *ortho* position. Further, we probed the electronic properties of

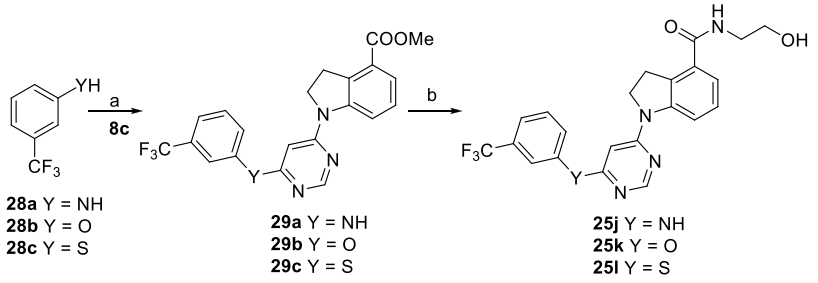
https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 5. Synthetic Routes of Compounds 25a–i[a]



[a]Reagents and conditions: (a) For **24a**, (i) **7a**, NaH, DMF, 0 °C to rt., and 2 h; (ii) *m*CPBA, 0 °C to rt., 10 min, and 47% for two steps; (iii) **6**, XantPhos, Cs_2CO_3, $Pd(OAc)_2$, 1,4-dioxane, 100 °C, overnight, and 82%; for **24b** and **24c**, (i) **8c**, NaH, DMF, 0 °C to rt., and 2 h; (ii) *m*CPBA, 0 °C to rt., 10 min, 49% over two steps for **24b**, and 79% over two steps for **24c**. (b) (i) Con. HCl/AcOH/H_2O, reflux, and overnight; (ii) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, and 63–77% for two steps. (c) $NaBH_4$, DMF/MeOH, 0 °C to rt., 30 min, and 68–90%. (d) (i) $NaBH_4$, DMF/MeOH, 0 °C to rt., and 30 min; (ii) con. HCl/AcOH/H_2O, reflux, overnight, and 78–90%. (e) (i) DAST, CH_2Cl_2, 0 °C to rt., and 10 min; (ii) THF/H_2O, reflux, overnight, and 60–76% for two steps. (f) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, and 68–86%.

Scheme 6. Synthetic Routes of Compounds 25j–l[a]

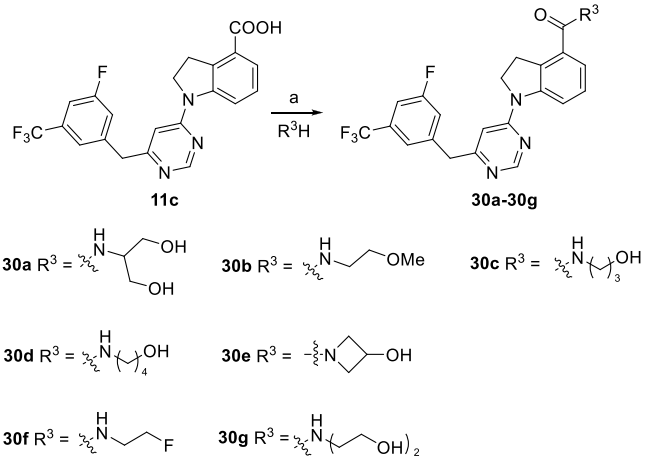


[a]Reagents and conditions: (a) For **29a**, $Pd(OAc)_2$, XantPhos, Cs_2CO_3, 1,4-dioxnae, 100 °C, overnight, and 69%; for **29b** and **29c**, Cs_2CO_3, DMF, 120 °C, overnight, 63% for **29b**, and 71% for **29c**. (b) (i) Con. HCl/AcOH/H_2O, reflux, and overnight; (ii) $NH_2CH_2CH_2OH$, EDCI, DMAP, DMF, rt., overnight, and 43–73% for two steps.

substituents on the benzene ring of moiety C. Replacement of *meta*-CF_3 of **23a** with a methyl group led to compound **23d**, which displayed the best potency among this series with an EC_{50} of 90 nM while maintaining excellent efficacy with an E_{max} of 144% (Table 2 and Figure 3B). Similarly, compound **23e** was obtained by substitution of *meta*-CF_3 of **23a** with a F atom, which displayed no change in potency, an EC_{50} of 106 nM, and maintained excellent efficacy, an E_{max} of 148%, (Table 2). Next, we probed whether a second additional electron-withdrawing moiety in the 5-position would further improve potency and efficacy. Disubstituted variants of **23d** and **23e**, leading to compounds **23f** and **23g**, respectively, displayed

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX–XXX

Scheme 7. Synthetic Routes of Compounds 30a−g[a]

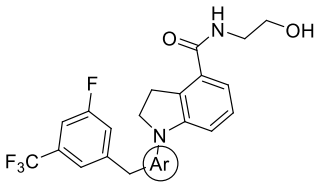


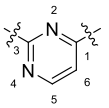
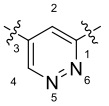
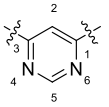
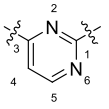
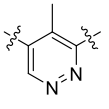
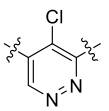
[a]Reagents and conditions: (a) EDCI, DMAP, DMF, rt., overnight, and 37−78%.

identical pharmacologic profiles as their monosubstituted counter parts (Table 2 and Figure 3B). These data suggest that the addition of an extra electron-withdrawing substituent at the 5-position of the benzene ring C has limited influence on the potency and efficacy. However, substitution of *meta*-CF_3 of **23a** with an electron-donating group such as OCF_3 (**23h**), OMe (**23i**), and 3,5-di-OMe (**23j**) dramatically decreased the potency as well as the efficacy (Table 2 and Figure 3B). Moreover, compound **23k** was synthesized without any substituent on the benzene ring C, resulting in ∼3-fold loss of potency relative to **23a**, suggesting that an electron-withdrawing substituent (e.g., **23a**, **23c**, and **23e**) on the benzene ring of moiety C is favorable for the potency. Taken together, these findings suggest that electron-withdrawing or small alkyl substituents at the 3-position are favorable for improved potency, with an additional small substituent at the 5-position also well-tolerated, while compounds without any substituent on the benzene ring or with electron-donating groups are not tolerated.

To probe the impact of the flexibility and steric availability around the linker between moieties B and C, compounds **25a−l** were designed, synthesized, and evaluated, with the results summarized in Table 3. We first replaced the methylene linker with a carbonyl moiety to yield compounds **25a−c**, which were

Table 1. EC_{50} and E_{max} of Compounds 12a−f

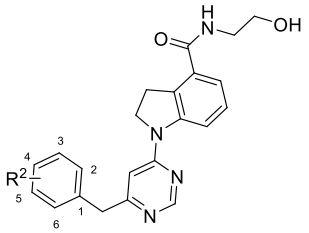


Compound	Ar	ClogP[a]	EC_{50} (nM)[b]	E_{max} (%)[b]
4		4.11	119 ± 18	100 ± 5
12a		3.64	373 ± 35	144 ± 7
12b		3.64	158 ± 48	158 ± 12
12c		3.64	135 ± 16	136 ± 6
12d		3.64	186 ± 44	138 ± 8
12e		3.95	754 ± 62	119 ± 6
12f		4.30	562 ± 80	97 ± 7

[a]cLogP: http://biosig.unimelb.edu.au/pkcsm/prediction. [b]The values are the mean ± SEM of at least three independent experiments. E_{max} (%) is the efficacy maximum of the compounds in the cAMP assay relative to compound **4** as 100% and 0.5% DMSO as 0%.

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 2. EC_{50} and E_{max} of Compounds 23a–k



compound	R^2	ClogP[a]	EC_{50} (nM)[b]	E_{max} (%)[b]
4		4.11	119 ± 18	100 ± 5
23a	3-CF_3	3.50	101 ± 31	127 ± 10
23b	2-CF_3	3.50	711 ± 160	115 ± 6
23c	4-CF_3	3.50	109 ± 19	136 ± 7
23d	3-Me	2.79	90 ± 19	144 ± 9
23e	3-F	2.62	106 ± 35	148 ± 14
23f	3,5-di-Me	3.10	97 ± 20	140 ± 11
23g	3,5-di-F	2.76	115 ± 19	142 ± 10
23h	3-OCF_3	3.38	131 ± 24	113 ± 11
23i	3-OMe	2.49	351 ± 6	122 ± 8
23j	3,5-di-OMe	2.50	850 ± 35	113 ± 2
23k	H	2.48	292 ± 17	134 ± 4

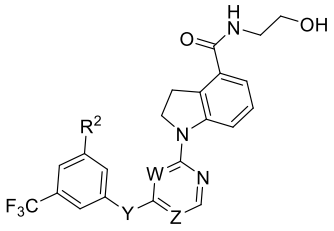
[a]cLogP: http://biosig.unimelb.edu.au/pkcsm/prediction. [b]The values are the mean ± SEM of at least three independent experiments. E_{max} (%) is the efficacy maximum of the compounds in the cAMP assay relative to compound **4** as 100% and 0.5% DMSO as 0%.

found to dramatically decrease the potency and efficacy, likely due to limited flexibility. Reduction of the carbonyl moiety of compounds **25a–c** produced compounds **25d–f**, and this restored a certain degree of the potency and efficacy but still at least 3-fold less potency than compound **23a**. Fluorination of compounds **25d–f** yielded compounds **25g–i**, which failed to improve the potency or efficacy (Table 3 and Figure 3C). Replacement of the methylene linker of compound **23a** with either an NH, O, or S led to corresponding compounds **25j–l**; however, none of these three compounds showed favorable potency and efficacy (Table 3 and Figure 3C). Taken together, these results suggest that GPR52 ligands of this scaffold need to have a flexible and sterically limited linker to achieve good potency. These SAR results are consistent with the recently disclosed cocrystal structure of compound **2** and GPR52, suggesting that the GPR52 binding region for these molecules is a narrow sterically limited pocket.[12]

Finally, to detect the impact of an amino alcohol side chain in moiety A, compounds with various lengths and flexibilities were designed through different strategies, including introducing extra hydroxyl groups (**30a** and **30g**), modification of the terminal hydroxyl group (**30b** and **30f**), and changing the length of the alkyl linker between amino and hydroxyl groups (**30c–e**) (Figure 3D). Unfortunately, these seven compounds substantially lost GPR52 potency compared to compound **12c** (Table 4), indicating that moiety A is critical and unlikely amenable for further optimization.

Molecular Docking Study of Compound 12c with GPR52. To understand the potential binding mode and the receptor–ligand interactions that are important for agonist activity at GPR52, molecular docking using Schrödinger Drug Discovery Suite was employed. Interestingly, the cocrystal structure of GPR52 bound to compound **2** was recently solved (PDB code: 6LI0), affording an unprecedented opportunity to pursue computational docking studies with an orphan GPCR.[12] Compound **12c** was selected as the example of our optimized compounds to further explore the binding interactions with GPR52. As shown in Figure 4, the docking results indicated that compound **12c** has energetically favorable interactions with the narrow pocket surrounded by ECL2, TM1, TM2, and TM7 and forms a similar binding pose to compound **2** with GPR52 in the solved cocrystal structure.[12] Notably, there are three critical hydrogen bonding interactions

Table 3. EC_{50} and E_{max} of Compounds 25a–l

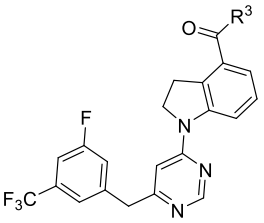


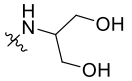
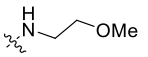
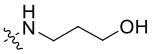
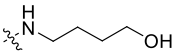
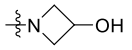
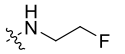
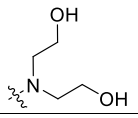
compound	R^2	Y	W	Z	ClogP[a]	EC_{50} (nM)[b]	E_{max} (%)[b]
4					4.11	119 ± 18	100 ± 5
25a	F	CO	N	CH	3.28	1589 ± 166	96 ± 7
25b	F	CO	CH	N	3.28	1693 ± 437	22 ± 3
25c	H	CO	CH	N	3.14	1008 ± 237	22 ± 4
25d	F	CHOH	N	CH	3.13	399 ± 68	130 ± 9
25e	F	CHOH	CH	N	3.13	338 ± 26	134 ± 28
25f	H	CHOH	CH	N	2.99	560 ± 19	89 ± 4
25g	F	CHF	N	CH	4.10	329 ± 113	144 ± 9
25h	F	CHF	CH	N	4.10	330 ± 38	85 ± 5
25i	H	CHF	CH	N	3.97	557 ± 273	83 ± 5
25j	H	NH	CH	N	3.66	1105 ± 24	81 ± 2
25k	H	O	CH	N	3.70	2404 ± 292	99 ± 4
25l	H	S	CH	N	4.06	371 ± 47	123 ± 6

[a]cLogP: http://biosig.unimelb.edu.au/pkcsm/prediction. [b]The values are the mean ± SEM of at least three independent experiments. E_{max} (%) is the efficacy maximum of the compounds in the cAMP assay relative to compound **4** as 100% and 0.5% DMSO as 0%.

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX–XXX

Table 4. EC_{50} and E_{max} of Compounds 30a−g



Compound	R^3	ClogP[a]	EC_{50} (nM)[b]	E_{max} (%)[b]
4		4.11	119 ± 18	100 ± 5
30a		3.00	346 ± 19	115 ± 15
30b		4.30	489 ± 86	119 ± 7
30c		4.03	275 ± 13	120 ± 15
30d		4.42	399 ± 51	120 ± 18
30e		3.73	760 ± 176	111 ± 10
30f		4.62	431 ± 7	129 ± 17
30g		3.35	673 ± 135	119 ± 15

[a]cLogP: http://biosig.unimelb.edu.au/pkcsm/prediction. [b]The values are the mean ± SEM of at least three independent experiments. E_{max} (%) is the efficacy maximum of the compounds in the cAMP assay relative to compound **4** as 100% and 0.5% DMSO as 0%.

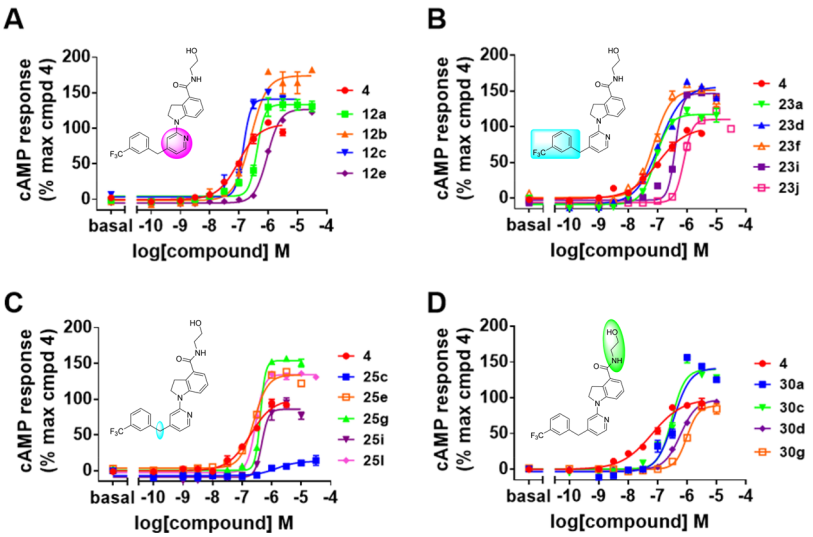


Figure 3. Concentration responses of select GPR52 agonists on cAMP signaling in HEK293 cells. HEK293 cells expressing human GPR52 and the cAMP Glosensor reporter were subjected to twelve-point concentration response (0.3 nM−30 μM) testing. (A) Modifications around ring B (magenta): comparison with compounds **4**, **12a**, **12b**, **12c**, and **12e**. (B) Modifications around ring C (cyan): comparison with compounds **4**, **23a**, **23d**, **23f**, **23i**, **23j**. (C) Modifications around carbon linker (cyan): comparison with compounds **4**, **25c**, **25e**, **25g**, **25i**, and **25l**. (D) Modifications around head group A (green): comparison with compounds **4**, **30a**, **30c**, **30d**, and **30g**. All results are presented as the mean ± SEM from triplicate testing in a representative experiment, with similar results observed in 3−17 experiments.

between compound **12c** and the residues of the ECL2 and TM1 of the receptor: the **12c** terminal hydroxyl group forms hydrogen bonds with residues Glu191 and Ile189 of ECL2, and the **12c** carbonyl group of the amide forms a hydrogen bond with residue Cys40 from TM1. This binding model may explain the significant activity loss when replacing the amino alcohol side chain into other side chains. The pyrimidine ring (core ring B) forms a $\pi-\pi$ stacking interaction with the residue Phe300 of TM7. Moreover, the benzene ring (moiety C) of compound **12c** pointed into a narrow hydrophobic pocket and formed hydrophobic interactions with residues Phe117, Cys94, Tyr185, Ile47, Ile121, Ile307, and Trp304. This may explain

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

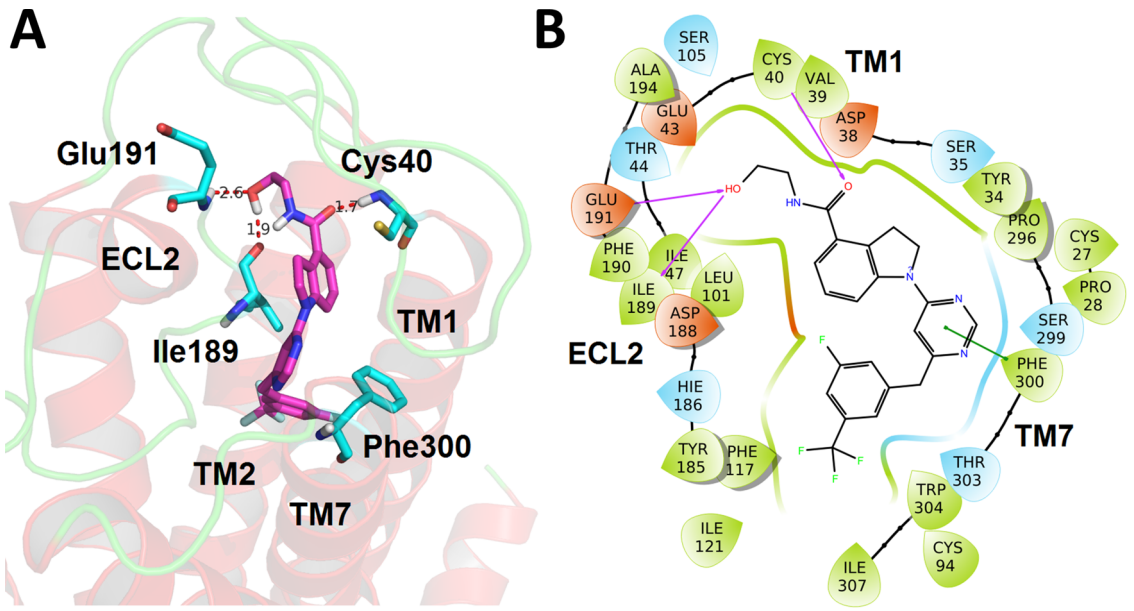


Figure 4. Putative binding mode and molecular docking of compound **12c** with GPR52 (PDB code: 6LI0). (A) Docking of compound **12c** (magenta) into the binding pocket of GPR52. Important residues are drawn as sticks. Hydrogen bonds are shown as dashed red lines. (B) Docking of compound **12c** into the binding pocket of GPR52 in 2D view. Hydrogen bonds are shown as magenta lines, and $\pi-\pi$ interaction is shown as a green line.

why modifications with only small substitutions on moiety C are tolerated, and why the hydrophobic groups (e.g., F and CF_3) are more favorable for the binding to drive potency. This docking also indicates that the binding pocket near ECL2 is a hot spot of interaction for both previously identified GPR52 agonists as well as **12c** and likely other indoline-carboxamide-based ligands.

***In Vitro* Assessment of Off-Target Effects of Compound 12c.** Considering the potential drug-like properties of chemical scaffolds and overall *in vitro* activities of GPR52 activation, compound **12c** was selected as a representative chemical probe among the identified active analogs to pursue further pharmacological evaluations in a proof-of-concept study. To evaluate if the cAMP signaling activity of optimized compound **12c** is indeed due to GPR52 agonist activation, **12c** was further tested in control studies using HEK293 cells lacking GPR52. Results determined that **12c** did not increase cAMP in the cells lacking GPR52 (Supporting Information, Figure S2). Therefore, compound **12c** regulates cAMP increases through a direct interaction with GPR52 in this cellular system. To determine the selectivity of **12c** against other GPCRs, a broad-panel counter screening was tested by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP).[26] This testing determined that compound **12c** at a concentration of 10 μM displayed no significant binding affinity (K_i) at over 30 brain receptors or channels, including important GPCRs that are current targets of antipsychotic medications (e.g., 5-HT_{2A} and D_2 receptors), as shown in Table 5. In addition, compound **12c** at 10 μM did not show any human ether-a-go-go-related gene (hERG) potassium channel binding activity (Table 5), indicating that compound **12c** should not have undesirable hERG inhibition known to cause cardiotoxicity.[27]

***In Vivo* PK Profile of Compound 12c.** Based on its potency and efficacy as well as potential drug-like properties, compound **12c** was selected as the representative compound for further PK evaluations. Compound **12c** was evaluated in rats after a single dose of 20 mg/kg by oral (PO) or 10 mg/kg by intravenous (IV) administration, and the results are summarized in Table 6. Compound **12c** has excellent plasma exposure after PO ($AUC_{0\text{-inf}}$ = 13,749 ng·h/mL) and IV dosing ($AUC_{0\text{-inf}}$ = 9030 ng·h/mL), as well as high maximum serum concentration following PO (C_{max} = 3407 ng/mL) and IV administration (C_{max} = 6726 ng/mL). Additionally, compound **12c** displayed good volume of plasma distribution (V_{ss} = 1.5 L/kg) and acceptable plasma clearance (CL = 1.1 L/h/kg)

Table 5. Broad-Panel Counter Screening of 12c against Other GPCRs and Transporters[a]

GPCRs	% inhibition (10 μM)[b]	K_i (μM)[b]	GPCRs, transporters, ion channels	% inhibition (10 μM)	K_i (μM)[b]
5-HT1A	8.68	ND	D1	12.29	ND
5-HT1B	6.3	ND	D2	2.51	ND
5-HT1D	29.64	ND	D3	10.64	ND
5-HT1E	7.71	ND	D4	29.91	ND
5-HT2A	10.62	ND	D5	−7.17	ND
5-HT2B	28.69	ND	DAT	24.83	ND
5-HT2C	52.62	>10	GABAA	5.98	ND
5-HT3	26.82	ND	H1	6.43	ND
5-HT5A	13.95	ND	H2	22.36	ND
5-HT6	7.98	ND	KOR	1.48	ND
5-HT7A	5.16	ND	M1	2.86	ND
Alpha1A	13.56	ND	M2	−1.87	ND
Alpha1B	−5.42	ND	M3	45.38	ND
Alpha2A	4.45	ND	M4	29.26	ND
Beta1	17.2	ND	M5	0.82	ND
Beta2	−14.07	ND	MOR	16.24	ND
			hERG	11.04	ND

[a]The broad-panel counter screening of **12c** against a panel of GPCRs and transporters was generously provided by the NIMH Psychoactive Drug Screening Program. [b]The values are the mean percent inhibition of binding from at least three independent experiments, SEM < 20%. "ND" means that a K_i binding affinity was not detected.

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 6. *In Vivo* Pharmacokinetic Parameters of Compound 12c in Rats[a]

route	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	C_{max} (ng/mL)	CL (L/h/kg)	V_{ss} (L/kg)	*F* (%)
PO	13,749 ± 2710	2.5 ± 0.2	3407 ± 179	1.5 ± 0.3	5.5 ± 1.16	76 ± 15
IV	9030 ± 1018	1.0 ± 0.1	6726 ± 727	1.1 ± 0.1	1.5 ± 0.2	

[a]$AUC_{0-\infty}$, area under the curve (t = 0 to 24 h); $t_{1/2}$, terminal half-life; C_{max}, maximum concentration of the drug in plasma; CL, plasma clearance; V_{ss}, volume of distribution at steady state; *F*, absolute oral bioavailability. Experiments were studied in biological triplicates, and data values are shown as the mean ± SEM.

after 10 mg/kg IV. Excellent oral bioavailability (*F*) with the value of 76% was observed. Compound **12c** was evaluated in rats for brain permeability and concentration after a single dose of 10 mg/kg by IV administration, and the concentrations of **12c** in the brain and plasma were measured at 0.25 and 1 h after administration. As shown in Table 7, compound **12c** exhibits a good concentration in the brain at 0.25 h with the value of 1807 ng/g and also exhibits an acceptable brain permeability with a brain/plasma ratio of 0.28. The high brain concentration and brain permeability of **12c** persisted for at least 1 h, with a brain concentration over 1000 ng/g and a brain/plasma ratio of 0.39 after 1 h injection. Based upon these collective findings, compound **12c** was selected for further *in vivo* evaluation.

Table 7. Brain Permeability of Compound 12c in Rats[a]

route	time (h)	brain conc. (ng/g)	plasma conc. (ng/g)	brain/plasma ratio
IV	0.25	1807 ± 198	6324 ± 271	0.28 ± 0.02
	1	1006 ± 43	2589 ± 241	0.39 ± 0.02

[a]Concentrations of compound **12c** in the brain and plasma were determined at 0.25 and 1 h after a single dose of 10 mg/kg by IV. Experiments were studied in biological triplicates, and data values are shown as the mean ± SEM.

Efficacy Evaluation of Compound 12c *In Vivo*. Compound **12c** exhibited potent GPR52 activity *in vitro* and a good PK profile to support further *in vivo* assessment. Amphetamine-induced hyperlocomotion in rodents is a well-characterized task with high translational validity for predicting preclinical antipsychotic-like activity.[28,29] Given that GPR52 agonists inhibit hyperactivity induced by psychostimulants,[10,30] we employed this assay to investigate the efficacy of compound **12c** *in vivo*. Mice were injected intraperitoneally (IP) with a vehicle or compound **12c** (0.3, 1, 3, or 10 mg/kg in vehicle; Figure 5A); automated activity monitoring began immediately and continued for 30 min. Mice were then injected with amphetamine (AMPH; 3 mg/kg IP), and activity was monitored for the next 90 min (Figure 5A). A one-way ANOVA indicated that compound **12c** suppressed horizontal activity ($F_{4,54}$ = 3.287, P < 0.05); Dunnett's *a priori* comparisons indicated that 3 mg/kg (P < 0.05) and 10 mg/kg (P < 0.05) suppressed horizontal activity (Figure 5B). Additionally, compound **12c** suppressed AMPH-induced horizontal activity ($F_{4,54}$ = 4.736, P < 0.05; Figure 5C) at both 3 mg/kg (P < 0.05) and 10 mg/kg doses (P < 0.05). Taken together, compound **12c** is an orally bioavailable and brain-penetrant GPR52 agonist, which dose-dependently inhibits amphetamine-evoked hyperactivity, suggesting therapeutic potential for neuropsychiatric diseases.

CONCLUSIONS

To understand the key ligand−receptor interactions and identify potent and efficacious GPR52 agonists, we have conducted a comprehensive structural optimization campaign based on previously reported advanced chemical lead **4** through a systematic SAR study by introduction of various amino alcohol side chains and modifications of numerous substituents on the core B ring and C ring. Among them, several compounds **12c** (**PW0787**), **23a** (**PW0860**), **23d** (**PW0878**), **23e** (**PW0885**), **23f** (**PW0888**), and **23h** (**PW0890**) were identified as novel GPR52 agonists that elevated cAMP signaling and were more potent than reference compound **4**. Compound **12c** has a favorable ClogP and a good *in vivo* PK profile. Therefore, compound **12c** was selected for further *in vivo* efficacy determination. Molecular docking studies of compound **12c** and GPR52 suggest a binding mode with three critical hydrogen bond pairs, $\pi-\pi$ stacking with ring B, and hydrophobic interactions with ring C. This binding model offers theoretical studies for further SAR studies of compound **12c**. Compound **12c** was also counter screened and displayed no off-target affinities at other important brain GPCRs and ion channels. In addition, compound **12c** has excellent oral plasma exposure, maximum serum concentration, bioavailability, and brain concentration. *In vivo* studies showed that compound **12c** significantly inhibited amphetamine-induced hyperactivity in mice. Therefore, newly discovered GPR52 agonist **12c** provides a useful pharmacological tool for studying GPR52 functions and for evaluating GPR52 agonist therapeutic potential for the treatment of brain diseases such as neuropsychiatric disorders.

EXPERIMENTAL SECTION

General. All commercially available starting materials and solvents were reagent grade and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063−0.200 mm (70−230 mesh, flash). Analytical TLC was carried out by employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Bruker-600 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts downfield from TMS were expressed in ppm, and *J* values were given in Hz. High-resolution mass spectra (HRMS) were obtained from a Thermo Fisher LTQ Orbitrap Elite mass spectrometer. Parameters include the following: nano ESI spray voltage was 1.8 kV, capillary temperature was 275 °C, and the resolution was 60,000; ionization was achieved by positive mode. Purity of final compounds was determined by analytical HPLC, which was carried out on a Shimadzu HPLC system (model: CBM-20A LC-20AD SPD-20A UV/vis). HPLC analysis conditions were a Waters μBondapak C18 (300 mm × 3.9 mm), a flow rate 0.5 mL/min, UV detection at 270 and 254 nm, and linear gradient from 10% acetonitrile in water (0.1% TFA) to 100% acetonitrile (0.1% TFA) in 20 min followed by 30 min of the last-named solvent. Compounds **4** and **6** were resynthesized in-house following reported synthetic procedures.[16,22] All biologically evaluated compounds are >95% pure.

Methyl 1-(2-Chloropyrimidin-4-yl)indoline-4-carboxylate (8a). To a solution of **6** (354 mg, 2 mmol) and 2,4-dichloropyrimidine (**7a**) (300 mg, 2 mmol) in EtOH (5 mL) was

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

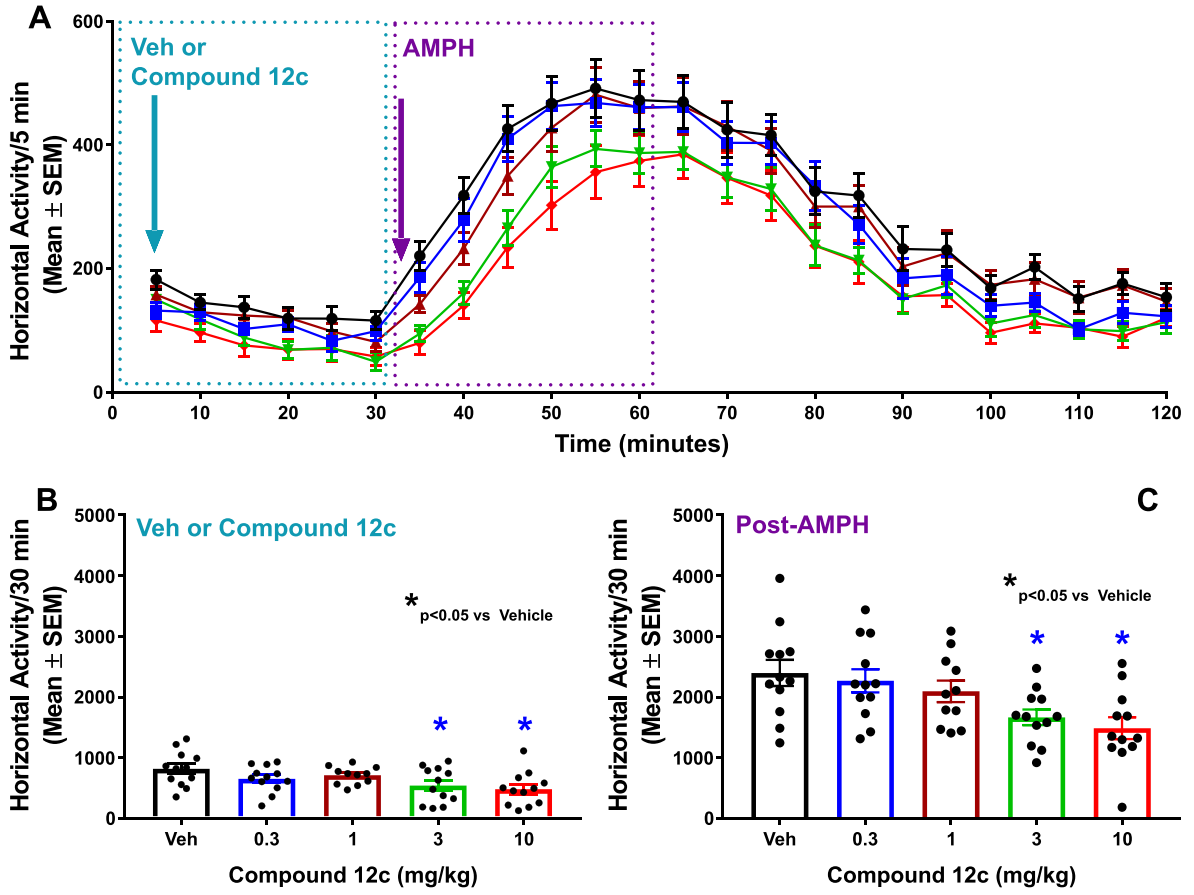


Figure 5. Compound **12c** shows antipsychotic-like activity in mice. (A) Time course of horizontal activity (counts/5 min; mean ± SEM) following injection of the vehicle (black circles) or compound **12c** (0.3 (blue squares), 1 (burgundy triangles), 3 (green inverted triangles), or 10 mg/kg IP (red diamonds) is shown during automated activity monitoring for 30 min (blue dotted box)). Mice were then injected with amphetamine (AMPH; 3 mg/kg IP), and activity was monitored for the next 90 min. Horizontal activity (counts/5 min; mean ± SEM) during the first 30 min following AMPH injection is illustrated (purple dotted box). (B) Bar graph showing horizontal activity/30 min (mean ± SEM) following injection of the vehicle (Veh) or compound **12c**. (C) Bar graph showing horizontal activity (mean ± SEM) in the first 30 min period after AMPH injection. Filled circles indicate horizontal activity for individual animals. n = 12 mice/dose except for the 1 mg/kg AMPH (n = 11 mice/dose). *P < 0.05 *vs* Veh.

added DIPEA (387 mg, 3 mmol) at rt., and the mixture solution was stirred at rt. overnight. After the reaction was completed (detected by TLC), the white solid was filtered and washed with water and cold EtOH. The cake was collected and dried to afford the product as white solid **8a** (387 mg, 67%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (d, J = 8.1 Hz, 1H), 8.34 (d, J = 6.0 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 6.1 Hz, 1H), 4.08 (t, J = 8.6 Hz, 2H), 3.85 (s, 3H), 3.50 (s, 2H).

Methyl 1-(5-Chloropyridazin-3-yl)indoline-4-carboxylate (8b). Compound **8b** (364 mg, 63%) was synthesized by a procedure similar to that used to prepare compound **8a** as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.68 (d, J = 8.2 Hz, 1H), 7.55−7.47 (m, 2H), 7.37 (s, 1H), 4.11 (t, J = 8.7 Hz, 2H), 3.86 (s, 3H), 3.53 (t, J = 8.7 Hz, 2H).

Methyl 1-(6-Chloropyrimidin-4-yl)indoline-4-carboxylate (8c). Compound **8c** (370 mg, 64%) was synthesized by a procedure similar to that used to prepare compound **8a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (dd, J = 8.2, 1.1 Hz, 1H), 8.61 (d, J = 0.8 Hz, 1H), 7.56 (dd, J = 7.9, 1.0 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 6.97 (d, J = 0.9 Hz, 1H), 4.07 (t, J = 8.5 Hz, 2H), 3.85 (s, 3H), 3.50 (t, J = 8.5 Hz, 2H).

Methyl 1-(2-(Cyano(3-fluoro-5-(trifluoromethyl)phenyl)-methyl)pyrimidin-4-yl)indoline-4-carboxylate (10a). 2-(3-Fluoro-5-(trifluoromethyl)phenyl)acetonitrile (**9a**) (336 mg, 1.6 mmol) was dissolved in 5 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. NaH (141 mg, 3.5 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at 0 °C for 30 min. Then, **8a** (480 mg, 1.6 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at rt. for 2 h. After the reaction was completed (detected by TLC), the reaction was quenched with $NH_4Cl_{(sat.\ aq.)}$. The solution was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. The residue was purified by silica gel chromatography (gradient: 1 to 5% MeOH in CH_2Cl_2) and provided product **10a** (300 mg, 66%) as a yellow solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.67 (d, J = 8.3 Hz, 1H), 8.40 (d, J = 6.1 Hz, 1H), 7.71 (dd, J = 8.0, 1.0 Hz, 2H), 7.54 (dt, J = 8.8, 2.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 2H), 6.53 (d, J = 6.1 Hz, 1H), 5.40 (s, 1H), 4.06 (dd, J = 9.3, 7.8 Hz, 2H), 3.94 (s, 3H), 3.66 (t, J = 8.5 Hz, 2H).

Methyl 1-(5-(Cyano(3-fluoro-5-(trifluoromethyl)phenyl)-methyl)pyridazin-3-yl)indoline-4-carboxylate (10b). Compound **10b** (120 mg, 53%) was synthesized by a procedure similar to that used to prepare compound **10a** as a yellow solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.74−8.62 (m, 2H), 7.66 (dd, J = 7.9, 1.0 Hz, 1H), 7.48 (s, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.33 (ddd, J = 8.1, 4.9, 3.0 Hz, 2H), 7.01 (dd, J = 1.9, 0.8 Hz, 1H), 5.25 (s, 1H), 4.14 (t, J = 8.6 Hz, 2H), 3.94 (s, 3H), 3.69 (t, J = 8.5 Hz, 2H).

Methyl 1-(6-(Cyano(3-fluoro-5-(trifluoromethyl)phenyl)-methyl)pyrimidin-4-yl)indoline-4-carboxylate (10c). Compound **10c** (130 mg, 57%) was synthesized by a procedure similar

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

to that used to prepare compound **10a** as a yellow solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.83−8.70 (m, 2H), 7.71 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.60 (s, 1H), 7.48 (d, *J* = 8.7 Hz, 1H), 7.36 (t, *J* = 8.1 Hz, 2H), 6.80 (s, 1H), 5.22 (s, 1H), 4.19−4.07 (m, 2H), 3.95 (s, 3H), 3.69 (t, *J* = 8.5 Hz, 2H).

1-(2-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-indoline-4-carboxylic acid (11a). To a solution of **10a** (300 mg, 0.7 mmol) in con. HCl (4 mL), H_2O (1 mL) and AcOH (1 mL) were added, and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as light yellow solid **11a** (256 mg, 93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.12 (s, 1H), 8.62−8.51 (m, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.83−7.55 (m, 4H), 7.10−6.93 (m, 2H), 4.51 (s, 2H), 4.16 (t, *J* = 8.4 Hz, 2H), 3.52 (t, *J* = 8.3 Hz, 4H).

1-(5-(3-Fluoro-5-(trifluoromethyl)benzyl)pyridazin-3-yl)-indoline-4-carboxylic acid (11b). Compound **11b** (101 mg, 92%) was synthesized by a procedure similar to that used to prepare compound **11a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (d, *J* = 1.4 Hz, 1H), 8.48 (d, *J* = 8.1 Hz, 1H), 7.74−7.69 (m, 2H), 7.69−7.63 (m, 1H), 7.57 (dd, *J* = 10.7, 7.6 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 1H), 4.23 (s, 2H), 4.17 (t, *J* = 8.5 Hz, 2H), 3.54 (s, 2H).

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-indoline-4-carboxylic Acid (11c). Compound **11c** (100 mg, 91%) was synthesized by a procedure similar to that used to prepare compound **11a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.71 (d, *J* = 8.2 Hz, 1H), 7.73 (s, 1H), 7.71−7.65 (m, 2H), 7.62 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.21 (s, 1H), 4.28 (s, 2H), 4.21 (t, *J* = 8.3 Hz, 2H), 3.59 (t, *J* = 8.3 Hz, 2H).

1-(2-(3-Fuoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (12a). Compound **11a** (42 mg, 0.1 mmol) and 2-aminoethan-1-ol (13 mg, 0.2 mmol) were dissolved in 2 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. HOBt (14 mg, 0.1 mmol), EDCI (39 mg, 0.2 mmol), and DMAP (24 mg, 0.2 mmol) were added to the solution at 0 °C. Then, the ice bath was removed, and the mixture solution was stirred at room temperature overnight. After the reaction was completed (detected by TLC), the reaction was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. This material was further purified by preparative TLC plates using CH_2Cl_2/MeOH = 50:1 as the eluent to yield **12a** as a white solid (33 mg, 73%). ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.26 (dd, *J* = 6.3, 2.6 Hz, 1H), 8.14 (dd, *J* = 7.9, 2.4 Hz, 1H), 7.45 (s, 1H), 7.29−7.04 (m, 5H), 6.39 (dd, *J* = 6.5, 2.5 Hz, 1H), 4.20 (s, 2H), 3.93 (t, *J* = 8.6 Hz, 2H), 3.71 (t, *J* = 5.0 Hz, 2H), 3.48 (qd, *J* = 8.7, 7.3, 2.6 Hz, 4H). ^{13}C NMR (75 MHz, chloroform-*d* and MeOD) δ 168.9, 167.3, 159.0, 155.9, 144.1, 142.0, 132.4, 130.9, 127.5, 122.4−122.1 (m), 120.3, 120.0, 110.8 (d, *J* = 24.5 Hz), 103.1, 61.2, 49.2, 45.0, 42.3, 27.5. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_2$, 461.1595; found, 461.1591.

1-(5-(3-Fluoro-5-(trifluoromethyl)benzyl)pyridazin-3-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (12b). Compound **12b** (35 mg, 76%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, methanol-d_4) δ 8.58 (s, 1H), 8.45 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.53 (s, 1H), 7.39 (tt, *J* = 6.3, 2.0 Hz, 2H), 7.29−7.17 (m, 2H), 7.11 (d, *J* = 1.6 Hz, 1H), 4.14 (s, 2H), 4.03 (t, *J* = 8.6 Hz, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 3.51 (t, *J* = 5.8 Hz, 2H), 3.45 (t, *J* = 8.6 Hz, 2H). ^{13}C NMR (75 MHz, MeOD) δ 169.6, 164.4, 161.1, 144.6, 142.7 (d, *J* = 7.7 Hz), 131.7, 131.6, 127.1, 121.6−121.4 (m), 119.7, 119.4, 116.7, 114.1, 111.1 (d, *J* = 4.1 Hz), 111.0, 110.7 (d, *J* = 4.0 Hz), 60.23, 48.81, 41.92, 37.30, 27.06. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_2$, 461.1595; found, 461.1591.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (12c). Compound **12c** (38 mg, 83%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.70 (s, 1H), 8.57 (d, *J* = 8.1 Hz, 1H), 7.37 (s, 1H), 7.30−7.16 (m, 3H), 7.10 (d, *J* = 7.7 Hz, 1H), 6.71 (t, *J* = 5.6 Hz, 1H), 6.33 (s, 1H), 4.04 (s, 2H), 3.91 (t, *J* = 8.6 Hz, 2H), 3.83 (t, *J* = 4.9 Hz, 2H), 3.60 (d, *J* = 5.1 Hz, 2H), 3.49 (d, *J* = 8.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 167.5, 166.7, 163.9, 160.7, 159.5, 157.9, 144.3, 143.8, 143.7, 132.6, 132.5, 131.2 (qd, *J* = 32.1, 8.3 Hz), 127.6, 122.5 (p, *J* = 3.6 Hz), 123.8 (qd, *J* = 270.8, 3.0 Hz), 121.1, 120.9, 120.6, 117.9, 111.4 (q, *J* = 4.0 Hz), 111.1 (q, *J* = 3.7 Hz), 105.0, 60.2, 48.9, 42.7, 42.4, 27.7. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_2$, 461.1595; found, 461.1591.

2-(2-Chloropyrimidin-4-yl)-2-(3-fluoro-5-(trifluoromethyl)-phenyl)acetonitrile (13). 2-(3-Fluoro-5-(trifluoromethyl)phenyl)-acetonitrile (**9a**) (406 mg, 2 mmol) was dissolved in 5 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. NaH (190 mg, 4.4 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at 0 °C for 30 min. Then, 2,4-dichloropyrimidine (**7a**) (450 mg, 3 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at rt. for 2 h. After the reaction was completed (detected by TLC), the reaction mixture was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. The residue was purified by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) to provide product **13** (460 mg, 73%) as a yellow oil. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.76 (d, *J* = 5.0 Hz, 1H), 7.57 (s, 1H), 7.52 (d, *J* = 5.0 Hz, 1H), 7.43 (ddd, *J* = 14.3, 7.2, 1.8 Hz, 2H), 5.30 (s, 1H).

2-Chloro-4-(3-fluoro-5-(trifluoromethyl)benzyl)pyrimidine (14). To a solution of **13** (460 mg, 1.5 mmol) in con. HCl (4 mL), H_2O (1 mL) and AcOH (1 mL) were added, and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as a light yellow solid. The yellow solid was dissolved in 2 mL of $POCl_3$, and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature. After the reaction was completed (detected by TLC), the reaction was poured into ice water, and the pH of the mixture solution was adjusted to pH = 8 with $Na_2CO_{3(sat.\ aq.)}$ solution. The solution was extracted with EtOAc (20 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) to provide product **14** as a brown colorless oil (222 mg, 51% for two steps). ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.57 (d, *J* = 5.0 Hz, 1H), 7.36 (s, 1H), 7.30−7.26 (m, 1H), 7.22 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.09 (d, *J* = 5.0 Hz, 1H), 4.17 (s, 2H).

Methyl 1-(4-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-2-yl)indoline-4-carboxylate (15). A mixture of **14** (145 mg, 0.5 mmol), $Pd(OAc)_2$ (6 mg, 0.025 mmol), XantPhos (29 mg, 0.05 mmol), Cs_2CO_3 (325 mg, 1 mmol), and **6** (89 mg, 0.5 mmol) in 1,4-dioxane (3 mL) was subjected to three rounds of vacuum evacuation followed by introduction of nitrogen. The reaction mixture was then stirred at 100 °C overnight. The reaction was cooled to room temperature and poured in water and then extracted with EtOAc (10 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) to provide product **15** (120 mg, 56%) as a yellow solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.52−8.42 (m, 2H), 7.60 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.45 (s, 1H), 7.26 (dd, *J* = 8.4, 3.5 Hz, 3H), 6.62 (d, *J* = 5.0 Hz, 1H), 4.28 (dd, *J* = 9.3, 8.2 Hz, 2H), 4.10 (s, 2H), 3.93 (s, 3H), 3.60−3.53 (m, 2H).

1-(4-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-2-yl)-indoline-4-carboxylic Acid (16). To a solution of **14** (120 mg, 0.28 mmol) in MeOH (4 mL) was added a 2 N solution of NaOH (1 mL), and the mixture solution was stirred at reflux for 1 h. The pH of the mixture solution was adjusted to pH = 1 with 2 N HCl solution, and then, a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

dried to afford the product as light yellow solid **16** (110 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (d, J = 5.0 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 7.67 (s, 1H), 7.59 (dt, J = 9.5, 2.0 Hz, 2H), 7.44 (dd, J = 7.8, 1.1 Hz, 1H), 7.13 (t, J = 8.0 Hz, 1H), 6.91 (d, J = 5.0 Hz, 1H), 4.23 (s, 2H), 4.14 (t, J = 8.7 Hz, 2H), 3.43 (t, J = 8.6 Hz, 2H).

1-(4-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-2-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (12d). Compound **12d** (92 mg, 83%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.37 (d, J = 5.0 Hz, 1H), 8.30 (dd, J = 7.4, 1.5 Hz, 1H), 7.43 (s, 1H), 7.24 (dd, J = 8.9, 1.4 Hz, 2H), 7.16−7.05 (m, 2H), 6.77 (t, J = 5.6 Hz, 1H), 6.57 (d, J = 5.0 Hz, 1H), 4.17 (t, J = 8.7 Hz, 2H), 4.05 (s, 2H), 3.81 (t, J = 4.9 Hz, 2H), 3.58 (d, J = 5.1 Hz, 2H), 3.40 (q, J = 9.6, 8.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 167.6, 164.1, 160.8, 159.0, 157.9, 144.6, 141.7 (d, J = 7.7 Hz), 132.6, 130.8, 127.5, 122.1 (dd, J = 7.1, 3.5 Hz), 120.1, 119.8, 119.2, 117.6, 111.3 (q, J = 3.9 Hz), 111.0 (q, J = 3.8 Hz), 110.6, 62.1, 49.0, 43.5, 42.6, 27.3. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_2$, 461.1595; found, 461.1591.

2-(3-Fluoro-5-(trifluoromethyl)phenyl)-2-(5-methyl-6-oxo-1-(tetrahydro-2*H*-pyran-2-yl)-1,6-dihydropyridazin-4-yl)-acetonitrile (17a). Compound **17a** (150 mg, 36%) was synthesized by a procedure similar to that used to prepare compound **10a** as a yellow oil. ^{1}H NMR (300 MHz, chloroform-d) δ 7.75 (d, J = 8.8 Hz, 1H), 7.46−7.37 (m, 2H), 7.27 (dt, J = 8.5, 2.3 Hz, 1H), 6.06 (dt, J = 10.6, 1.9 Hz, 1H), 5.30 (d, J = 6.2 Hz, 1H), 4.17−4.09 (m, 1H), 3.75 (ddd, J = 11.7, 9.0, 2.7 Hz, 1H), 2.18 (dd, J = 10.7, 4.2 Hz, 1H), 1.85−1.58 (m, 4H).

2-(5-Chloro-6-oxo-1-(tetrahydro-2*H*-pyran-2-yl)-1,6-dihydropyridazin-4-yl)-2-(3-fluoro-5-(trifluoromethyl)phenyl)-acetonitrile (17b). Compound **17b** (520 mg, 63%) was synthesized by a procedure similar to that used to prepare compound **10a** as a yellow oil. ^{1}H NMR (300 MHz, chloroform-d) δ 7.90 (d, J = 6.1 Hz, 1H), 7.51 (s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.39−7.33 (m, 1H), 6.07 (dt, J = 10.6, 2.7 Hz, 1H), 5.59 (d, J = 6.9 Hz, 1H), 4.13 (td, J = 6.9, 3.2 Hz, 1H), 3.77 (ddd, J = 11.5, 8.7, 3.0 Hz, 1H), 2.24−2.06 (m, 2H), 1.87−1.62 (m, 4H).

5-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methylpyridazin-3(2*H*)-one (18a). To a solution of **17a** (830 mg, 2 mmol) in con. HCl (8 mL), H_2O (2 mL) and AcOH (2 mL) were added, and the mixture solution was stirred at reflux overnight. After the reaction was completed (detected by TLC), the reaction was poured into ice water. The pH of the mixture solution was adjusted to pH = 8 with $Na_2CO_{3(sat.\ aq.)}$ solution. Then, the solution was extracted with EtOAc (20 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue purification by silica gel chromatography (gradient: 30 to 50% EtOAc in hexane) provided product **18a** (480 mg, 78%) as a white solid. ^{1}H NMR (300 MHz, chloroform-d) δ 11.67 (s, 1H), 7.58 (s, 1H), 7.25 (d, J = 7.0 Hz, 2H), 7.07−6.99 (m, 1H), 3.97 (s, 2H), 2.23 (s, 3H).

4-Chloro-5-(3-fluoro-5-(trifluoromethyl)benzyl)pyridazin-3(2*H*)-one (18b). Compound **18b** (410 mg, 83%) was synthesized by a procedure similar to that used to prepare compound **18a** as a yellow oil. ^{1}H NMR (300 MHz, chloroform-d) δ 7.65 (s, 1H), 7.38−7.23 (m, 3H), 7.17−7.10 (m, 1H), 4.12 (s, 2H).

3-Chloro-5-(3-fluoro-5-(trifluoromethyl)benzyl)-4-methylpyridazine (19a). A solution of **18a** (400 mg, 1.3 mmol) in $POCl_3$ (3 mL) was heated to reflux for 3 h. After the reaction was completed (detected by TLC), the reaction was cooled to room temperature and poured in ice water. The pH of the mixture solution was adjusted to pH = 8 with 2 N NaOH solution and then extracted with EtOAc (30 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue purification by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) provided product **19a** (350 mg, 83%) as a brown solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.83 (s, 1H), 7.29 (d, J = 9.7 Hz, 2H), 7.22 (s, 1H), 6.99 (d, J = 8.8 Hz, 1H), 4.12 (s, 2H), 2.39 (s, 3H).

3,4-Dichloro-5-(3-fluoro-5-(trifluoromethyl)benzyl)-pyridazine (19b). Compound **19b** (350 mg, 80%) was synthesized by a procedure similar to that used to prepare compound **19a** as a brown solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.87 (s, 1H), 7.35−7.29 (m, 2H), 7.16−7.06 (m, 1H), 4.23 (s, 2H).

Methyl 1-(5-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methylpyridazin-3-yl)indoline-4-carboxylate (20a). Compound **20a** (142 mg, 64%) was synthesized by a procedure similar to that used to prepare compound **15** as a brown solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.76 (s, 1H), 7.50 (dd, J = 7.9, 0.9 Hz, 1H), 7.27 (d, J = 7.9 Hz, 4H), 7.10 (q, J = 9.6, 8.7 Hz, 2H), 6.46 (d, J = 7.8 Hz, 1H), 4.27 (t, J = 8.2 Hz, 2H), 4.13 (s, 2H), 3.94 (s, 3H), 3.57 (t, J = 8.2 Hz, 2H), 2.18 (s, 3H).

Methyl 1-(4-Chloro-5-(3-fluoro-5-(trifluoromethyl)benzyl)-pyridazin-3-yl)indoline-4-carboxylate (20b). Compound **20b** (280 mg, 60%) was synthesized by a procedure similar to that used to prepare compound **15** as a white solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.71 (s, 1H), 7.59 (dd, J = 7.9, 1.0 Hz, 1H), 7.37−7.29 (m, 2H), 7.25−7.12 (m, 2H), 7.00 (dd, J = 8.0, 0.9 Hz, 1H), 4.34 (t, J = 8.3 Hz, 2H), 4.23 (s, 2H), 3.94 (s, 3H), 3.60 (t, J = 8.3 Hz, 2H).

1-(5-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methylpyridazin-3-yl)indoline-4-carboxylic Acid (21a). Compound **21a** (126 mg, 92%) was synthesized by a procedure similar to that used to prepare compound **16** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.91 (d, J = 1.8 Hz, 1H), 7.66−7.47 (m, 3H), 7.38 (d, J = 7.9 Hz, 1H), 7.14 (t, J = 7.9 Hz, 1H), 6.64 (d, J = 7.9 Hz, 1H), 4.29 (s, 2H), 4.12 (t, J = 8.3 Hz, 2H), 3.45 (t, J = 8.0 Hz, 2H), 2.20 (d, J = 1.9 Hz, 3H).

1-(4-Chloro-5-(3-fluoro-5-(trifluoromethyl)benzyl)-pyridazin-3-yl)indoline-4-carboxylic Acid (21b). Compound **21a** (243 mg, 90%) was synthesized by a procedure similar to that used to prepare compound **16** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.00 (s, 1H), 7.65−7.51 (m, 3H), 7.44 (dd, J = 7.8, 1.0 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 6.95 (dd, J = 8.0, 1.0 Hz, 1H), 4.34 (s, 2H), 4.21 (t, J = 8.3 Hz, 4H), 3.46 (t, J = 8.2 Hz, 2H).

1-(5-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methylpyridazin-3-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (12e). Compound **12e** (107 mg, 79%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.71 (s, 1H), 7.29 (d, J = 9.7 Hz, 1H), 7.24 (s, 1H), 7.07 (d, J = 9.0 Hz, 1H), 7.02−6.84 (m, 3H), 6.29 (dd, J = 5.4, 3.4 Hz, 1H), 4.25−4.08 (m, 4H), 3.86 (t, J = 4.8 Hz, 2H), 3.63 (q, J = 5.1 Hz, 2H), 3.43 (t, J = 8.1 Hz, 3H), 2.15 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 158.9, 149.1, 147.8, 140.9 (d, J = 7.3 Hz), 138.7, 132.0, 131.8, 131.2, 126.8, 121.0 (t, J = 3.3 Hz), 119.3, 119. 0, 118.4, 111.9, 62.1, 53.6, 42.8, 36.0, 28.8, 14.5. HRMS (ESI): $(M + H)^+$ calcd for $C_{24}H_{23}F_4N_4O_2$, 475.1752; found, 475.1748.

1-(4-Chloro-5-(3-fluoro-5-(trifluoromethyl)benzyl)-pyridazin-3-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (12f). Compound **12f** (29 mg, 59%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d) δ 8.66 (s, 1H), 7.36−7.24 (m, 2H), 7.14 (d, J = 9.0 Hz, 1H), 7.11−7.02 (m, 2H), 6.89 (dd, J = 6.4, 3.6 Hz, 2H), 4.34−4.15 (m, 4H), 3.81 (t, J = 4.9 Hz, 2H), 3.60 (t, J = 5.1 Hz, 2H), 3.47 (t, J = 8.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 164.4, 161.0, 156.8, 148.2, 146.2, 139.9 (d, J = 7.4 Hz), 138.7, 132.1, 131.6, 130.2, 126.8, 121.5−121.4 (m), 119.6, 119.4, 119.3, 115.3, 112.2 (d, J = 3.6 Hz), 111.9 (d, J = 3.9 Hz), 62.1, 53.6, 42.7, 36.1, 29.1. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{20}ClF_4N_4O_2$, 495.1205; found, 495.1201.

1-(6-(3-(Trifluoromethyl)benzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22a). 2-(3-(Trifluoromethyl)phenyl)acetonitrile (**9b**) (370 mg, 2 mmol) was dissolved in 5 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. NaH (176 mg, 4.4 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at 0 °C for 30 min. Then, **8c** (580 mg, 2 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at rt. for 2 h. After the reaction was completed (detected by TLC), the reaction mixture was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow solid. ^{1}H NMR (300 MHz,

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

DMSO-d_6) δ 8.75 (s, 1H), 8.67 (d, J = 8.0 Hz, 1H), 7.93−7.85 (m, 2H), 7.77 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.54 (dd, J = 7.9, 1.0 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.07 (s, 1H), 6.00 (s, 1H), 4.08 (dd, J = 8.8, 2.0 Hz, 2H), 3.85 (s, 3H), 3.51 (t, J = 8.6 Hz, 2H).

To a solution of the yellow solid (100 mg, 0.23 mmol) in con. HCl (2 mL), H_2O (0.5 mL) and AcOH (0.5 mL) were added, and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as light yellow solid **22a** (82 mg, 90% for two steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.70 (d, J = 8.1 Hz, 1H), 7.81 (s, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.68−7.53 (m, 3H), 7.40 (t, J = 8.0 Hz, 1H), 7.13 (s, 1H), 4.26−4.11 (m, 4H), 3.57 (t, J = 8.4 Hz, 2H).

1-(6-(2-(Trifluoromethyl)benzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22b). Compound **22b** (65 mg, 54% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.70 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 7.7 Hz, 1H), 7.64 (dd, J = 10.4, 7.6 Hz, 2H), 7.55 (d, J = 7.4 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 8.1 Hz, 1H), 6.77 (s, 1H), 4.32 (s, 2H), 4.07 (t, J = 8.4 Hz, 2H), 3.53 (t, J = 8.4 Hz, 2H).

1-(6-(4-(Trifluoromethyl)benzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22c). Compound **22c** (68 mg, 57% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.69−8.66 (m, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.64−7.59 (m, 3H), 7.40 (d, J = 8.2 Hz, 1H), 7.08 (s, 1H), 4.19−4.12 (m, 4H), 3.54 (d, J = 8.7 Hz, 2H).

1-(6-(3-Methylbenzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22d). Compound **22d** (86 mg, 83% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.70 (d, J = 8.1 Hz, 1H), 7.81 (s, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.68−7.53 (m, 3H), 7.40 (t, J = 8.0 Hz, 1H), 7.13 (s, 1H), 4.26−4.11 (m, 4H), 3.57 (t, J = 8.4 Hz, 2H), 2.49 (s, 3H).

1-(6-(3-Fluorobenzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22e). Compound **22e** (71 mg, 68% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.71 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.47−7.38 (m, 2H), 7.31−7.23 (m, 2H), 7.16−7.06 (m, 2H), 4.24−4.13 (m, 4H), 3.58 (t, J = 8.4 Hz, 2H).

1-(6-(3,5-Dimethylbenzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22f). Compound **22f** (88 mg, 81% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.74 (d, J = 5.7 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.58 (s, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.17 (s, 1H), 7.02 (s, 2H), 4.24 (d, J = 8.1 Hz, 2H), 4.08 (s, 2H), 3.60 (d, J = 8.4 Hz, 2H), 2.25 (s, 6H).

1-(6-(3,5-Difluorobenzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22g). Compound **22g** (79 mg, 72% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.72 (d, J = 8.2 Hz, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.29−7.11 (m, 4H), 4.20 (d, J = 8.3 Hz, 4H), 3.58 (t, J = 8.3 Hz, 2H).

1-(6-(3-(Trifluoromethoxy)benzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22h). Compound **22h** (87 mg, 70% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.71 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.50 (d, J = 4.7 Hz, 3H), 7.43 (t, J = 8.0 Hz, 1H), 7.33−7.27 (m, 1H), 7.20 (s, 1H), 4.25−4.16 (m, 4H), 3.58 (t, J = 8.1 Hz, 2H).

1-(6-(3-Methoxybenzyl)pyrimidin-4-yl)indoline-4-arboxylic Acid (22i). Compound **22i** (90 mg, 83% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.70 (d, J = 8.0 Hz, 1H), 7.66 (dt, J = 7.8, 1.2 Hz, 2H), 7.45−7.38 (m, 2H), 7.13 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 7.1 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 4.13−4.04 (m, 4H), 3.75 (s, 3H), 3.58 (d, J = 8.2 Hz, 2H).

1-(6-(3,5-Dimethoxybenzyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (22j). Compound **22j** (94 mg, 80% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.12 (s, 1H), 8.94 (s, 1H), 8.71 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.43 (t, J = 8.1 Hz, 1H), 7.15 (d, J = 9.7 Hz, 1H), 6.44 (d, J = 19.6 Hz, 2H), 6.24 (d, J = 11.6 Hz, 1H), 4.20 (t, J = 8.4 Hz, 2H), 4.02 (s, 2H), 3.71 (d, J = 14.4 Hz, 6H), 3.58 (t, J = 8.2 Hz, 2H).

1-(6-Benzylpyrimidin-4-yl)indoline-4-carboxylic Acid (22k). Compound **22k** (76 mg, 77% for two steps) was synthesized by a procedure similar to that used to prepare compound **22a** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.19 (s, 1H), 8.96 (s, 1H), 8.71 (d, J = 8.2 Hz, 1H), 7.70 (dd, J = 7.9, 1.0 Hz, 1H), 7.48−7.42 (m, 2H), 7.41−7.27 (m, 3H), 7.17 (s, 1H), 4.25−4.16 (m, 4H), 3.57 (t, J = 8.2 Hz, 2H).

***N*-(2-Hydroxyethyl)-1-(6-(3-(trifluoromethyl)benzyl)-pyrimidin-4-yl)indoline-4-carboxamide (23a).** Compound **23a** (52 mg, 59%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.62 (s, 1H), 8.48 (d, J = 7.6 Hz, 1H), 7.52−7.37 (m, 4H), 7.21−7.09 (m, 2H), 6.32 (s, 1H), 4.01 (s, 2H), 3.87 (t, J = 8.6 Hz, 2H), 3.70 (t, J = 5.2 Hz, 2H), 3.53−3.37 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.8, 166.7, 159.4, 157.5, 144.2, 138.6, 132.5, 132.4, 131.1 (d, J = 5.2 Hz), 131.0, 129.2, 127.7, 125.9−125.6 (m), 123.7 (d, J = 3.9 Hz), 120.3, 118.6, 103.9, 61.1, 48.7, 43.5, 42.2, 27.4. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{22}F_3N_4O_2$, 443.1689; found, 443.1685.

***N*-(2-Hydroxyethyl)-1-(6-(2-(trifluoromethyl)benzyl)-pyrimidin-4-yl)indoline-4-carboxamide (23b).** Compound **23b** (36 mg, 61%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.65 (s, 1H), 8.48 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.34 (d, J = 8.1 Hz, 2H), 7.21 (ddd, J = 28.3, 13.0, 6.9 Hz, 3H), 6.19 (s, 1H), 4.18 (s, 2H), 3.83 (t, J = 8.5 Hz, 2H), 3.70 (t, J = 5.3 Hz, 2H), 3.53−3.39 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.9, 166.9, 159.4, 157.3, 144.3, 135.7, 132.4, 132.1, 131.0, 127.7, 127.1, 126.2 (d, J = 5.6 Hz), 120.2, 118.5, 104.0, 61.1, 48.6, 42.3, 40.1, 27.4. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{22}F_3N_4O_2$, 443.1689; found, 443.1685.

***N*-(2-Hydroxyethyl)-1-(6-(4-(trifluoromethyl)benzyl)-pyrimidin-4-yl)indoline-4-carboxamide (23c).** Compound **23c** (31 mg, 53%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.65 (s, 1H), 8.52 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.38 (d, J = 7.9 Hz, 2H), 7.27−7.12 (m, 3H), 6.35 (s, 1H), 4.03 (s, 2H), 3.91 (t, J = 8.6 Hz, 2H), 3.72 (t, J = 5.2 Hz, 2H), 3.56−3.41 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.9, 166.8, 159.4, 157.5, 144.3, 141.8, 132.4, 131.0, 129.4, 127.7, 125.6 (q, J = 3.7 Hz), 120.3, 118.6, 104.0, 61.2, 48.7, 43.6, 42.3, 27.5. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{22}F_3N_4O_2$, 443.1689; found, 443.1685.

***N*-(2-Hydroxyethyl)-1-(6-(3-methylbenzyl)pyrimidin-4-yl)-indoline-4-carboxamide (23d).** Compound **23d** (43 mg, 83%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.63 (s, 1H), 8.49 (d, J = 7.9 Hz, 1H), 7.34−7.09 (m, 4H), 7.03 (d, J = 7.7 Hz, 3H), 6.33 (s, 1H), 3.99−3.82 (m, 4H), 3.71 (t, J = 5.1 Hz, 2H), 3.56−3.34 (m, 5H), 2.30 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.9, 168.1, 159.4, 157.3, 144.4, 138.3, 137.5, 132.4, 131.0, 129.8, 128.6, 127.7, 127.5, 126.1, 120.2, 118.5, 103.9, 61.2, 49.1, 43.8, 42.2, 27.4, 21.2. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{25}N_4O_2$, 389.1972; found, 389.1968.

1-(6-(3-Fluorobenzyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)-indoline-4-carboxamide (23e). Compound **23e** (38 mg, 72%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.68−8.57 (m, 1H), 8.51 (d, J = 7.8 Hz, 1H), 7.32−7.11 (m, 4H), 7.02 (d, J = 7.7 Hz, 1H), 6.98−6.86 (m, 2H), 6.34 (s, 1H), 4.02−3.86 (m, 4H), 3.71 (t, J = 5.2 Hz, 2H), 3.55−3.40 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.9, 168.9, 167.1, 164.5, 161.2, 159.5,

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

157.4, 144.3, 140.0 (d, J = 7.4 Hz), 132.4, 131.0 (2C), 130.2 (d, J = 8.3 Hz), 127.7, 124.8 (d, J = 2.9 Hz), 120.3, 118.6, 115.9 (d, J = 21.4 Hz), 113.73 (d, J = 21.0 Hz), 103.9, 61.1, 48.7, 43.5, 43.4, 42.2, 27.5. HRMS (ESI): $(M + H)^+$ calcd for $C_{22}H_{22}FN_4O_2$, 393.1721; found, 393.1716.

1-(6-(3,5-Dimethylbenzyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (23f). Compound **23f** (42 mg, 78%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.59 (s, 1H), 8.46 (d, J = 7.9 Hz, 1H), 7.40−7.05 (m, 3H), 6.82 (s, 3H), 6.32 (s, 1H), 3.87 (d, J = 5.0 Hz, 4H), 3.73−3.56 (m, 4H), 3.46 (d, J = 6.6 Hz, 2H), 2.23 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 169.0, 168.1, 159.4, 157.2, 144.3, 138.2, 137.4, 132.3, 131.0, 128.4, 127.6, 126.8, 120.2, 118.4, 103.9, 61.0, 48.7, 43.7, 42.3, 27.4, 21.1 (2C). HRMS (ESI): $(M + H)^+$ calcd for $C_{24}H_{27}N_4O_2$, 403.2129; found, 403.2125.

1-(6-(3,5-Difluorobenzyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (23g). Compound **23g** (37 mg, 67%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.81−8.40 (m, 2H), 7.63−7.05 (m, 3H), 6.96−6.55 (m, 3H), 6.43 (s, 1H), 3.97 (d, J = 9.2 Hz, 4H), 3.89−3.66 (m, 4H), 3.54 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 172.9, 170.1, 168.6 (d, J = 13.3 Hz), 165.4, 163.4, 161.4, 148.1, 145.4, 136.3, 135.1, 131.7, 124.4, 122.6, 116.0, 115.7, 108.0, 106.6, 106.2, 105.9, 65.0, 52.7, 47.2, 46.2, 46.1, 31.4. HRMS (ESI): calcd for $C_{22}H_{21}F_2N_4O_2$, 411.1627; found, 411.1623.

***N*-(2-Hydroxyethyl)-1-(6-(3-(trifluoromethoxy)benzyl)-pyrimidin-4-yl)indoline-4-carboxamide (23h).** Compound **23h** (41 mg, 67%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, methanol-d_4) δ 8.57 (d, J = 3.4 Hz, 2H), 7.43 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.29−7.22 (m, 3H), 7.20−7.13 (m, 1H), 6.65 (s, 1H), 4.05 (s, 2H), 3.94 (t, J = 8.6 Hz, 2H), 3.74 (t, J = 5.8 Hz, 2H), 3.51 (t, J = 5.8 Hz, 2H), 3.41 (t, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, MeOD) δ 169.4, 166.6, 159.6, 156.9, 149.3, 144.0, 140.7, 132.1, 131.6, 129.9, 127.6, 127.1, 121.4, 120.6, 118.8, 118.4, 104.0, 60.2, 48.4, 42.5, 41.9, 26.9. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{22}F_3N_4O_3$, 459.1639; found, 459.1636.

***N*-(2-Hydroxyethyl)-1-(6-(3-methoxybenzyl)pyrimidin-4-yl)-indoline-4-carboxamide (23i).** Compound **23i** (26 mg, 48%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.72 (s, 1H), 8.59 (d, J = 8.2 Hz, 1H), 7.28−7.19 (m, 2H), 7.05 (d, J = 7.7 Hz, 1H), 6.95−6.79 (m, 3H), 6.51 (t, J = 5.4 Hz, 1H), 6.36 (s, 1H), 4.01 (s, 2H), 3.94 (t, J = 8.6 Hz, 2H), 3.87 (t, J = 4.9 Hz, 2H), 3.82 (s, 3H), 3.63 (q, J = 5.3 Hz, 2H), 3.54 (t, J = 8.5 Hz, 2H), 2.91 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 168.0, 159.9, 159.4, 157.5, 144.7, 139.4, 132.6, 130.7, 129.7, 127.8, 121.6, 119.6, 118.6, 115.1, 112.1, 103.8, 62.4, 55.2, 48.7, 44.3, 42.7, 27.7. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{25}N_4O_3$, 405.1921; found, 405.1917.

1-(6-(3,5-Dimethoxybenzyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (23j). Compound **23j** (17 mg, 29%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.67 (d, J = 1.0 Hz, 1H), 8.55 (d, J = 8.1 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 6.98 (dd, J = 7.8, 1.0 Hz, 1H), 6.56 (t, J = 5.6 Hz, 1H), 6.48 (d, J = 2.3 Hz, 2H), 6.39 (t, J = 2.3 Hz, 1H), 6.32 (d, J = 1.1 Hz, 1H), 3.94 (s, 2H), 3.91 (d, J = 8.5 Hz, 2H), 3.87−3.83 (m, 2H), 3.80 (s, 6H), 3.62 (t, J = 5.1 Hz, 2H), 3.50 (t, J = 8.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 167.6, 161.0, 159.3, 157.4, 144.6, 140.0, 132.5, 130.7, 127.7, 119.7, 118.5, 107.4, 103.8, 98.6, 62.2, 55.4 (2C), 48.7, 44.5, 42.7, 27.6. HRMS (ESI): $(M + H)^+$ calcd for $C_{24}H_{27}N_4O_4$, 435.2027; found, 435.2024.

1-(6-Benzylpyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (23k). Compound **23k** (39 mg, 78%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.60 (s, 1H), 8.47 (d, J = 7.9 Hz, 1H), 7.33−7.07 (m, 8H), 6.27 (s, 1H), 3.96 (s, 2H), 3.82 (t, J = 8.5 Hz, 2H), 3.71 (t, J = 5.1 Hz, 2H), 3.49 (q, J = 5.3 Hz, 2H), 3.44−3.30 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 167.9, 166.6, 159.3, 157.2, 144.3, 137.6, 132.3, 131.0, 129.1, 128.7, 127.6, 126.8, 120.2, 118.5, 103.9, 61.2, 48.6, 43.8, 42.3, 27.4. HRMS (ESI): $(M + H)^+$ calcd for $C_{22}H_{23}N_4O_2$, 375.1816; found, 375.1812.

Methyl 1-(4-(3-Fluoro-5-(trifluoromethyl)benzoyl)-pyrimidin-2-yl)indoline-4-carboxylate (24a). Compound **9a** (406 mg, 2 mmol) was dissolved in 5 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. NaH (190 mg, 4.4 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at 0 °C for 30 min. Then, 2,4-dichloropyrimidine (450 mg, 3 mmol) was added to the solution at 0 °C. After the mixture solution was stirred at rt. for 2 h, the mixture was cooled to 0 °C with an ice bath. Followed by adding *m*CPBA (516 mg, 3 mmol), the mixture solution was stirred at 0 °C for 30 min. After the reaction was completed (detected by TLC), the reaction was worked up by the addition of water and EtOAc extraction. The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. The residue purification by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) provided product (2-chloropyrimidin-4-yl)(3-fluoro-5-(trifluoromethyl)phenyl)methanone (280 mg, 47%) as a yellow solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.98 (d, J = 4.9 Hz, 1H), 8.30 (s, 1H), 8.14 (dt, J = 8.9, 1.9 Hz, 1H), 7.96 (d, J = 4.9 Hz, 1H), 7.63 (dt, J = 8.1, 2.0 Hz, 1H).

A mixture of (2-chloropyrimidin-4-yl)(3-fluoro-5-(trifluoromethyl)phenyl)methanone (91 mg, 0.3 mmol), $Pd(OAc)_2$ (3 mg, 0.015 mmol), XantPhos (17 mg, 0.03 mmol), Cs_2CO_3 (196 mg, 0.6 mmol), and **6** (53 mg, 0.3 mmol) in 1,4-dioxane (3 mL) was subjected to three rounds of vacuum evacuation followed by introduction of nitrogen. The reaction mixture was then stirred at 100 °C overnight. The reaction mixture was cooled to room temperature and poured into water and then extracted with EtOAc (10 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue purification by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) provided product **24a** (110 mg, 82%) as a yellow solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.84 (d, J = 4.8 Hz, 1H), 8.43 (s, 2H), 8.15 (d, J = 8.8 Hz, 1H), 7.64 (dd, J = 7.9, 1.2 Hz, 2H), 7.39 (d, J = 4.8 Hz, 1H), 7.23 (t, J = 8.0 Hz, 1H), 4.32 (t, J = 8.7 Hz, 2H), 3.94 (s, 3H), 3.62 (t, J = 8.7 Hz, 2H).

Methyl 1-(6-(3-Fluoro-5-(trifluoromethyl)benzoyl)-pyrimidin-4-yl)indoline-4-carboxylate (24b). 2-(3-Fluoro-5-(trifluoromethyl)phenyl)acetonitrile (**9a**) (406 mg, 2 mmol) was dissolved in 5 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. NaH (190 mg, 4.4 mmol) was added to the solution at 0 °C, and the mixture solution was stirred at 0 °C for 30 min. Then, compound **8c** (580 mg, 2 mmol) was added to the solution at 0 °C. After the mixture solution was stirred at rt. for 2 h, the mixture was cooled to 0 °C with an ice bath. Followed by adding *m*CPBA (516 mg, 3 mmol), the mixture solution was stirred at 0 °C for 10 min. After the reaction was completed (detected by TLC), the reaction was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. The residue purification by silica gel chromatography (gradient: 20 to 50% EtOAc in hexane) provided product **24b** (440 mg, 49%) as a yellow solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.97 (d, J = 1.1 Hz, 1H), 8.82 (d, J = 8.1 Hz, 1H), 8.31 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.73 (dd, J = 7.9, 1.0 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.39 (t, J = 8.0 Hz, 1H), 7.31 (s, 1H), 4.21 (t, J = 8.6 Hz, 2H), 3.96 (s, 3H), 3.72 (t, J = 8.6 Hz, 2H).

Methyl 1-(6-(3-(Trifluoromethyl)benzoyl)pyrimidin-4-yl)-indoline-4-carboxylate (24c). Compound **24c** (162 mg, 79%) was synthesized by a procedure similar to that used to prepare compound **24b** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (d, J = 0.9 Hz, 1H), 8.67 (d, J = 8.1 Hz, 1H), 7.94−7.84 (m, 2H), 7.77 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.54 (dd, J = 7.9, 1.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.08 (s, 1H), 6.01 (s, 1H), 4.10 (t, J = 9.8 Hz, 2H), 3.85 (s, 3H), 3.51 (t, J = 8.5 Hz, 2H).

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

1-(4-(3-Fluoro-5-(trifluoromethyl)benzoyl)pyrimidin-2-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25a). To a solution of **24a** (45 mg, 0.1 mmol) in con. HCl (4 mL), H_2O (1 mL) and AcOH (1 mL) were added, and the mixture solution was stirred at reflux overnight. The reaction mixture was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as a light yellow solid. The yellow solid and 2-aminoethan-1-ol (13 mg, 0.2 mmol) were dissolved in 2 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. HOBt (14 mg, 0.1 mmol), EDCI (39 mg, 0.2 mmol), and DMAP (24 mg, 0.2 mmol) were added to the solution at 0 °C. Then, the ice bath was removed, and the mixture solution was stirred at room temperature overnight. After the reaction was completed (detected by TLC), the reaction was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. This material was further purified by preparative TLC plates using CH_2Cl_2/MeOH = 50:1 as the eluent to yield **25a** as a yellow solid (36 mg, 77% for two steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (d, J = 4.9 Hz, 1H), 8.33 (s, 1H), 8.17 (dd, J = 26.9, 8.6 Hz, 3H), 7.44 (d, J = 4.9 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 7.10 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 4.19 (t, J = 8.6 Hz, 2H), 3.51 (q, J = 6.1 Hz, 2H), 3.39 (t, J = 8.7 Hz, 2H), 3.30 (s, 2H). ^{13}C NMR (75 MHz, DMSO) δ 159.9, 143.8, 132.7, 127.4, 122.0, 120.9, 116.7, 110.5, 60.2, 49.4, 42.4, 42.3, 40.8, 27.3. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{19}F_4N_4O_3$, 475.1388; found, 475.1385.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzoyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25b). Compound **25b** (35 mg, 75%) was synthesized by a procedure similar to that used to prepare compound **25a** as a yellow solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.86 (d, J = 1.1 Hz, 1H), 8.63 (d, J = 7.7 Hz, 1H), 8.21 (s, 1H), 8.07 (dt, J = 8.8, 2.0 Hz, 1H), 7.55 (dt, J = 8.0, 2.0 Hz, 1H), 7.28−7.23 (m, 2H), 7.22−7.20 (m, 1H), 4.10 (t, J = 8.5 Hz, 2H), 3.74 (t, J = 5.1 Hz, 2H), 3.54 (dd, J = 9.3, 7.1 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 190.3, 168.8, 166.6, 163.7, 160.4, 159.5 (d, J = 59.6 Hz), 157.3, 143.9, 138.0, 132.7, 131.2, 127.8, 123.9−123.3 (m), 121.3 (d, J = 23.2 Hz), 120.9, 119.0, 117.34 (dd, J = 24.4, 3.2 Hz), 105.1, 61.2, 49.0, 42.3, 27.5. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{19}F_4N_4O_3$, 475.1388; found, 475.1386.

***N*-(2-Hydroxyethyl)-1-(6-(3-(trifluoromethyl)benzoyl)-pyrimidin-4-yl)indoline-4-carboxamide (25c).** Compound **25c** (360 mg, 63%) was synthesized by a procedure similar to that used to prepare compound **25a** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (d, J = 1.1 Hz, 1H), 8.63 (dd, J = 6.6, 2.6 Hz, 1H), 8.38−8.25 (m, 3H), 8.13−8.05 (m, 1H), 7.88−7.77 (m, 1H), 7.39−7.26 (m, 3H), 4.73 (t, J = 5.6 Hz, 1H), 4.17 (t, J = 8.5 Hz, 2H), 3.59−3.41 (m, 4H), 3.34 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 192.0, 167.4, 160.0, 159.9 (d, J = 8.6 Hz), 157.5, 143.9, 136.4, 135.0, 132.9 (d, J = 14.2 Hz), 132.7, 130.2, 129.4, 127.7, 127.3 (d, J = 3.6 Hz), 127.2, 121.8, 118.3, 105.5, 60.2, 49.0, 42.4, 27.7. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{20}F_3N_4O_3$, 457.1482; found, 457.1480.

1-(4-((3-Fluoro-5-(trifluoromethyl)phenyl)(hydroxy)-methyl)pyrimidin-2-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25d). To a solution of **25a** (10 mg, 0.02 mmol) in MeOH (1 mL) was added $NaBH_4$ (1 mg, 0.02 mmol) at 0 °C, and the mixture solution was stirred at rt. for 1 h. After the reaction was completed (detected by TLC), the reaction mixture was worked up by the addition of water and EtOAc extraction. The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a colorless oil. This material was further purified by preparative TLC plates using CH_2Cl_2/MeOH = 50:1 as the eluent to yield **25d** as a white solid (9 mg, 93%). ^{1}H NMR (300 MHz, chloroform-d) δ 8.50−8.35 (m, 2H), 7.57 (s, 1H), 7.37 (d, J = 9.1 Hz, 1H), 7.24 (dd, J = 15.9, 7.8 Hz, 2H), 7.12 (d, J = 7.7 Hz, 1H), 6.66 (t, J = 4.5 Hz, 2H), 5.67 (s, 1H), 4.94 (s, 1H), 4.24 (t, J = 8.8 Hz, 2H), 3.85 (t, J = 4.9 Hz, 2H), 3.63 (q, J = 5.2 Hz, 2H), 3.44 (t, J = 8.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 168.7, 164.2, 160.9, 158.4, 158.1, 145.8 (d, J = 6.9 Hz), 144.4, 132.9 (d, J = 8.1 Hz), 132.5 (d, J = 8.0 Hz), 130.9, 127.7, 125.0 (d, J = 3.0 Hz), 121.3 (d, J = 3.0 Hz), 119.5−119.24 (m), 117.7, 117.3 (d, J = 22.2 Hz), 112.5 (ddd, J = 24.2, 7.1, 3.4 Hz), 108.0, 73.8, 62.3, 49.2, 42.6, 27.2. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_3$, 477.1544; found, 477.1540.

1-(6-((3-Fluoro-5-(trifluoromethyl)phenyl)(hydroxy)-methyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25e). Compound **25e** (32 mg, 78%) was synthesized by a procedure similar to that used to prepare compound **25d** as a white solid. ^{1}H NMR (300 MHz, chloroform-d and MeOD) δ 8.66−8.27 (m, 2H), 7.51 (s, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.26−7.01 (m, 3H), 6.68 (s, 1H), 5.60 (s, 1H), 3.90 (t, J = 8.6 Hz, 2H), 3.70 (s, 2H), 3.46−3.27 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.7 (d, J = 36.5 Hz), 164.0, 159.4, 156.8, 146.2, 144.1, 132.4, 131.0, 127.6, 120.5, 119.2, 118.7, 117.2 (d, J = 22.5 Hz), 112.4−111.7 (m), 101.0, 73.9, 61.1, 42.3, 42.2, 27.3. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_3$, 477.1544; found, 477.1540.

1-(6-(Hydroxy(3-(trifluoromethyl)phenyl)methyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25f). Compound **25f** (41 mg, 90%) was synthesized by a procedure similar to that used to prepare compound **25d** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (s, 1H), 8.54 (d, J = 7.0 Hz, 1H), 8.25 (t, J = 5.8 Hz, 1H), 7.89−7.72 (m, 2H), 7.69−7.52 (m, 2H), 7.28 (d, J = 6.6 Hz, 2H), 7.09 (s, 1H), 6.45 (d, J = 4.5 Hz, 1H), 5.72 (d, J = 4.5 Hz, 1H), 4.71 (t, J = 5.6 Hz, 1H), 4.11 (q, J = 7.9 Hz, 2H), 3.60−3.33 (m, 6H). ^{13}C NMR (75 MHz, DMSO) δ 170.9, 167.5, 159.7, 157.4, 145.2, 144.4, 132.6, 131.5, 129.6, 129.2 (d, J = 31.4 Hz), 127.6, 126.5, 124.5 (t, J = 3.9 Hz), 123.7 (t, J = 3.9 Hz), 121.1, 117.9, 101.3, 74.5, 60.3, 49.0, 42.4, 27.7. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{22}F_3N_4O_3$, 459.1639; found, 459.1635.

1-(4-((3-Fluoro-5-(trifluoromethyl)phenyl)(hydroxy)-methyl)pyrimidin-2-yl)indoline-4-carboxylic Acid (26a). To a solution of **24a** (427 mg, 1 mmol) in DMF (5 mL) and MeOH (5 mL) was added $NaBH_4$ (38 mg, 1 mmol) at 0 °C, and the mixture solution was stirred at rt. for 30 min. After the reaction was completed (detected by TLC), the reaction mixture was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a colorless oil. The colorless oil was dissolved in con. HCl (4 mL), H_2O (1 mL), and AcOH (1 mL), and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as a light yellow solid (210 mg, 78%). ^{1}H NMR (300 MHz, chloroform-d) δ 8.63 (s, 1H), 8.51 (d, J = 4.8 Hz, 1H), 7.74 (dd, J = 7.9, 1.0 Hz, 1H), 7.57 (s, 1H), 7.36 (t, J = 10.4 Hz, 3H), 6.67 (d, J = 5.1 Hz, 1H), 5.73 (s, 1H), 4.39 (t, J = 8.3 Hz, 2H), 3.66 (t, J = 8.6 Hz, 2H).

1-(6-((3-Fluoro-5-(trifluoromethyl)phenyl)(hydroxy)-methyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (26b). Compound **26b** (390 mg, 90%) was synthesized by a procedure similar to that used to prepare compound **26a** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.71 (d, J = 7.8 Hz, 1H), 7.84 (s, 1H), 7.77 (d, J = 9.5 Hz, 1H), 7.64 (d, J = 7.8 Hz, 2H), 7.45−7.40 (m, 1H), 7.32 (d, J = 2.9 Hz, 1H), 6.00 (s, 1H), 4.24 (dt, J = 9.0, 3.9 Hz, 2H), 3.61−3.50 (m, 3H).

1-(6-(Hydroxy(3-(trifluoromethyl)phenyl)methyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (26c). Compound **26c** (117 mg, 68%) was synthesized by a procedure similar to that used to prepare compound **26a** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (d, J = 3.4 Hz, 1H), 8.71 (d, J = 7.8 Hz, 1H), 7.96 (s, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.73−7.58 (m, 3H), 7.42 (t, J = 8.0 Hz, 1H), 7.31 (d, J = 2.3 Hz, 1H), 5.99 (s, 1H), 4.32−4.14 (m, 2H), 3.58 (t, J = 8.2 Hz, 2H).

1-(4-(Fluoro(3-fluoro-5-(trifluoromethyl)phenyl)methyl)-pyrimidin-2-yl)indoline-4-carboxylic Acid (27a). To a solution of **26a** (210 mg, 0.6 mmol) in DCM (5 mL) was added DAST (322 mg, 1.8 mmol) at 0 °C, and the mixture solution was stirred at 0 °C for 10 min. After the reaction was completed (detected by TLC), the

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

reaction mixture was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. The residue was dissolved in THF (2 mL) and H_2O (2 mL), and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as light yellow solid **27a** (140 mg, 54% for two steps). ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.63 (d, J = 5.0 Hz, 1H), 8.49 (d, J = 8.2 Hz, 1H), 7.73−7.64 (m, 2H), 7.46 (d, J = 8.9 Hz, 1H), 7.40−7.29 (m, 2H), 7.04 (dd, J = 5.1, 1.7 Hz, 1H), 6.41 (d, J = 46.4 Hz, 1H), 4.28 (dd, J = 8.3, 3.1 Hz, 2H), 3.61 (t, J = 8.8 Hz, 2H).

1-(6-(Fluoro(3-fluoro-5-(trifluoromethyl)phenyl)methyl)-pyrimidin-4-yl)indoline-4-carboxylic Acid (27b). Compound **27b** (260 mg, 60%) was synthesized by a procedure similar to that used to prepare compound **27a** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.01 (s, 1H), 8.71 (d, J = 7.6 Hz, 2H), 7.80−7.71 (m, 3H), 7.56 (ddd, J = 7.8, 3.3, 1.2 Hz, 1H), 7.35 (dd, J = 9.1, 6.9 Hz, 1H), 7.11 (s, 1H), 6.75 (d, J = 45.7 Hz, 1H), 4.26−4.13 (m, 2H), 3.55 (t, J = 8.4 Hz, 2H).

1-(6-(Fluoro(3-(trifluoromethyl)phenyl)methyl)pyrimidin-4-yl)indoline-4-carboxylic Acid (27c). Compound **27c** (110 mg, 76%) was synthesized by a procedure similar to that used to prepare compound **27a** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.90 (s, 1H), 7.83−7.74 (m, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.56 (dd, J = 7.3, 3.1 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.10 (s, 1H), 6.72 (d, J = 45.7 Hz, 1H), 4.20 (dd, J = 9.4, 4.7 Hz, 2H), 3.55 (t, J = 8.6 Hz, 2H).

1-(4-(Fluoro(3-fluoro-5-(trifluoromethyl)phenyl)methyl)-pyrimidin-2-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25g). Compound **25g** (140 mg, 86%) was synthesized by a procedure similar to that used to prepare compound **12a** as a light yellow solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.54 (d, J = 5.0 Hz, 1H), 8.27 (d, J = 7.7 Hz, 1H), 7.63 (s, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.21−7.09 (m, 2H), 7.07−6.90 (m, 2H), 6.35 (d, J = 46.3 Hz, 1H), 4.17 (tt, J = 9.1, 3.5 Hz, 2H), 3.75 (t, J = 5.1 Hz, 2H), 3.54 (q, J = 5.3 Hz, 2H), 3.41 (t, J = 8.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 169.2, 169.1, 166.6 (d, J = 28.5 Hz), 164.0, 160.7, 159.0, 158.4 (d, J = 3.6 Hz), 144.3, 141.5 (dd, J = 21.8, 7.5 Hz), 132.7, 132.5, 131.0, 131.0, 127.5, 119.13−118.56 (m), 116.8 (dd, J = 22.9, 7.7 Hz), 113.2 (d, J = 23.1 Hz), 106.5 (d, J = 6.7 Hz), 91.95 (d, J = 179.2 Hz), 61.5, 49.1, 42.4, 27.2. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{20}F_5N_4O_2$, 479.1501; found, 479.1498.

1-(6-(Fluoro(3-fluoro-5-(trifluoromethyl)phenyl)methyl)-pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25h). Compound **25h** (162 mg, 68%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.74 (s, 1H), 8.66 (d, J = 8.1 Hz, 1H), 7.60 (s, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.37−7.31 (m, 1H), 7.28 (t, J = 4.0 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H), 6.86 (s, 1H), 6.61 (t, J = 5.8 Hz, 1H), 6.39 (d, J = 46.4 Hz, 1H), 4.11 (t, J = 8.6 Hz, 2H), 3.87 (q, J = 4.8 Hz, 2H), 3.72−3.53 (m, 4H), 2.73 (t, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 164.9 (d, J = 26.3 Hz), 164.1, 160.7, 159.6, 157.7 (d, J = 3.4 Hz), 144.4, 141.4, 132.8, 130.9, 127.9, 124.8, 121.2, 120.2, 119.1, 117.3 (d, J = 15.5 Hz), 113.4 (d, J = 23.9 Hz), 100.1 (d, J = 8.2 Hz), 92.07 (d, J = 179.4 Hz), 77.4, 62.3, 49.0, 42.6, 27.7. ^{19}F NMR (282 MHz, chloroform-*d*) δ −62.8, −109.5 (t, J = 8.7 Hz), −183.9 (d, J = 46.4 Hz). HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{20}F_5N_4O_2$, 479.1501; found, 479.1498.

1-(6-(Fluoro(3-(trifluoromethyl)phenyl)methyl)pyrimidin-4-yl)-*N*-(2-hydroxyethyl)indoline-4-carboxamide (25i). Compound **25i** (240 mg, 72%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.59 (dd, J = 15.6, 5.0 Hz, 2H), 7.76−7.42 (m, 4H), 7.18 (dt, J = 12.7, 7.0 Hz, 3H), 6.80 (d, J = 4.2 Hz, 1H), 6.35 (dd, J = 46.3, 4.4 Hz, 1H), 4.00 (q, J = 7.3 Hz, 2H), 3.74 (d, J = 5.6 Hz, 2H), 3.49 (dt, J = 20.7, 6.7 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 165.3 (d, J = 26.5 Hz), 159.5, 157.4, 144.1, 138.4 (d, J = 20.5 Hz), 132.5, 131.2, 131.1, 130.8, 130.3 (d, J = 6.5 Hz), 129.2, 127.7, 125.9, 123.5 (dt, J = 6.1, 2.8 Hz) 123.5, 120.5, 118.9, 100.2 (d, J = 8.1 Hz), 92.7 (d, J = 177.7 Hz), 61.3, 48.9, 42.4, 27.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ −62.8, −181.1. HRMS (ESI): $(M + H)^+$ calcd for $C_{23}H_{21}F_4N_4O_2$, 461.1595; found, 461.1590.

Methyl 1-(6-((3-(Trifluoromethyl)phenyl)amino)pyrimidin-4-yl)indoline-4-carboxylate (29a). A mixture of **28a** (48 mg, 0.3 mmol), $Pd(OAc)_2$ (3 mg, 0.015 mmol), XantPhos (17 mg, 0.03 mmol), Cs_2CO_3 (196 mg, 0.6 mmol), and **8c** (87 mg, 0.3 mmol) in 1,4-dioxane (3 mL) was subjected to three rounds of vacuum evacuation followed by introduction of nitrogen. The reaction mixture was then stirred at 100 °C overnight. The reaction mixture was cooled to room temperature and poured into water and then extracted with EtOAc (10 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography (gradient: 10 to 20% EtOAc in hexane) to provide product **29a** (86 mg, 69%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.65 (dd, J = 8.2, 1.1 Hz, 1H), 8.49 (s, 1H), 8.24 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.55−7.46 (m, 2H), 7.36−7.27 (m, 2H), 6.11 (s, 1H), 4.03 (t, J = 8.7 Hz, 2H), 3.86 (s, 3H), 3.52 (t, J = 8.7 Hz, 2H).

Methyl 1-(6-(3-(Trifluoromethyl)phenoxy)pyrimidin-4-yl)-indoline-4-carboxylate (29b). To a solution of **28b** (49 mg, 0.3 mmol) in DMF (2 mL) were added cesium carbonate (195 mg, 0.6 mmol) and **8c** (87 mg, 0.3 mmol), and the reaction was heated to 120 °C overnight. At this point, the reaction mixture was cooled to rt., poured into water (15 mL), and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with water (10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (eluent: 1% MeOH in CH_2Cl_2) and yielded product **29b** (79 mg, 63%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (t, J = 8.2 Hz, 1H), 8.47 (s, 1H), 7.73−7.50 (m, 5H), 7.36 (t, J = 8.2 Hz, 1H), 6.47 (d, J = 5.4 Hz, 1H), 4.10 (t, J = 8.5 Hz, 2H), 3.86 (s, 3H), 3.54 (t, J = 8.6 Hz, 2H).

Methyl 1-(6-((3-(Trifluoromethyl)phenyl)thio)pyrimidin-4-yl)indoline-4-carboxylate (29c). Compound **29c** (92 mg, 71%) was synthesized by a procedure similar to that used to prepare compound **29b** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63−8.50 (m, 2H), 8.01−7.85 (m, 3H), 7.75 (t, J = 7.8 Hz, 1H), 7.52 (dd, J = 7.9, 1.1 Hz, 1H), 7.32 (t, J = 8.1 Hz, 1H), 6.52 (d, J = 1.1 Hz, 1H), 3.91 (t, J = 8.6 Hz, 2H), 3.84 (s, 3H), 3.45 (t, J = 8.6 Hz, 2H).

***N*-(2-Hydroxyethyl)-1-(6-((3-(trifluoromethyl)phenyl)-amino)pyrimidin-4-yl)indoline-4-carboxamide (25j).** To a solution of **29a** (41 mg, 0.1 mmol) in con. HCl (4 mL), H_2O (1 mL) and AcOH (1 mL) were added, and the mixture solution was stirred at reflux overnight. The reaction was cooled to room temperature, and a yellow solid precipitated from the solution. The yellow solid was filtered and washed with water, and the cake was collected and dried to afford the product as a light yellow solid. The yellow solid and 2-aminoethan-1-ol (13 mg, 0.2 mmol) were dissolved in 2 mL of DMF, and the mixture solution was cooled to 0 °C with an ice bath. HOBt (14 mg, 0.1 mmol), EDCI (39 mg, 0.2 mmol), and DMAP (24 mg, 0.2 mmol) were added to the solution at 0 °C. Then, the ice bath was removed, and the mixture solution was stirred at rt. overnight. After the reaction was completed (detected by TLC), the reaction mixture was worked up by the addition of water and then extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered, and condensed by rotary evaporation to yield a yellow oil. This material was further purified by preparative TLC plates using CH_2Cl_2/MeOH = 50:1 as the eluent to yield **25j** as a white solid (43 mg, 73% for two steps). ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.43−8.25 (m, 2H), 7.66 (s, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.32−7.23 (m, 2H), 7.19−7.03 (m, 2H), 5.89 (s, 1H), 3.80 (t, J = 8.5 Hz, 2H), 3.70 (t, J = 5.2 Hz, 2H), 3.49 (t, J = 5.3 Hz, 2H), 3.34 (t, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 169.2, 166.6, 160.5, 159.7, 157.4, 153.0, 144.8, 139.9, 132.0, 131.6, 131.2, 131.0, 129.7, 127.6,

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

124.1, 119.6, 117.8, 117.5, 85.9, 61.1, 42.3, 27.4. HRMS (ESI): (M + H)$^+$ calcd for $C_{22}H_{21}F_3N_5O_2$, 444.1642; found, 444.1639.

***N*-(2-Hydroxyethyl)-1-(6-(3-(trifluoromethyl)phenoxy)-pyrimidin-4-yl)indoline-4-carboxamide (25k).** Compound **25k** (48 mg, 43%) was synthesized by a procedure similar to that used to prepare compound **25j** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.50 (d, J = 7.8 Hz, 1H), 8.39 (d, J = 3.5 Hz, 1H), 7.59−7.08 (m, 7H), 6.05 (d, J = 3.5 Hz, 1H), 3.93 (t, J = 8.8 Hz, 2H), 3.69 (q, J = 4.5 Hz, 2H), 3.48 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 169.3, 169.0, 161.2, 157.4, 152.9, 144.4, 132.3, 131.1, 130.3, 127.7, 124.9, 122.1, 120.3, 118.4, 89.3, 61.1, 42.3, 27.5. HRMS (ESI): (M + H)$^+$ calcd for $C_{22}H_{20}F_3N_4O_3$, 445.1482; found, 445.1480.

***N*-(2-Hydroxyethyl)-1-(6-((3-(trifluoromethyl)phenyl)thio)-pyrimidin-4-yl)indoline-4-carboxamide (25l).** Compound **25l** (64 mg, 56%) was synthesized by a procedure similar to that used to prepare compound **25j** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.47 (d, J = 3.9 Hz, 1H), 8.42−8.28 (m, 1H), 7.92−7.52 (m, 4H), 7.31−7.07 (m, 3H), 6.03 (d, J = 3.8 Hz, 1H), 3.71 (d, J = 5.7 Hz, 4H), 3.49 (t, J = 5.0 Hz, 2H), 3.40 (d, J = 9.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 169.0, 168.8, 158.5, 157.1, 144.1, 138.6, 132.4, 132.3, 131.9, 131.1, 130.2, 130.2, 127.6, 126.5, 120.4, 118.4, 100.4, 61.2, 48.6, 42.3, 42.2, 27.4. HRMS (ESI): (M + H)$^+$ calcd for $C_{22}H_{20}F_3N_4O_2S$, 461.1254; found, 461.1250.

***N*-(1,3-Dihydroxypropan-2-yl)-1-(6-(3-fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)indoline-4-carboxamide (30a).** Compound **30a** (28 mg, 57%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.68 (s, 1H), 8.55 (d, J = 7.5 Hz, 1H), 7.33 (s, 1H), 7.28−7.11 (m, 4H), 6.40 (s, 1H), 4.10−3.90 (m, 5H), 3.82 (dd, J = 11.3, 4.3 Hz, 2H), 3.70 (dd, J = 11.3, 5.1 Hz, 2H), 3.49 (t, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.5, 166.6, 166.0, 159.5, 157.7, 144.3, 141.6, 132.3, 130.9, 127.8, 121.8−121.5 (m), 120.5, 119.7, 119.4, 118.8, 111.5 (d, J = 3.9 Hz), 111.13 (d, J = 3.8 Hz), 104.0, 61.6, 52.6, 48.8, 43.4, 27.5. HRMS (ESI): (M + H)$^+$ calcd for $C_{24}H_{23}F_4N_4O_3$, 491.1701; found, 491.1697.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*-(2-methoxyethyl)indoline-4-carboxamide (30b). Compound **30b** (30 mg, 65%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.78 (s, 1H), 8.63 (d, J = 8.1 Hz, 1H), 7.38 (s, 1H), 7.31−7.21 (m, 3H), 7.17 (d, J = 7.7 Hz, 1H), 6.47 (d, J = 6.2 Hz, 1H), 6.43 (s, 1H), 4.08 (s, 2H), 4.02 (t, J = 8.6 Hz, 2H), 3.61 (ddd, J = 16.4, 10.7, 6.9 Hz, 6H), 3.42 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.6, 166.2, 164.2, 160.9, 159.5, 158.0, 144.5, 141.8 (d, J = 7.6 Hz), 132.9, 132.5, 132.5, 131.2, 127.8, 125.0, 121.7 (t, J = 3.6 Hz), 120.0, 119.6 (d, J = 21.4 Hz), 118.6, 111.3 (dd, J = 24.6, 3.9 Hz), 103.9, 77.4, 77.0, 76.6, 71.2, 48.8, 43.8, 39.5, 27.7. HRMS (ESI): (M + H)$^+$ calcd for $C_{24}H_{23}F_4N_4O_2$, 475.1752; found, 475.1749.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*-(3-hydroxypropyl)indoline-4-carboxamide (30c). Compound **30c** (26 mg, 56%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.77 (s, 1H), 8.63 (d, J = 8.1 Hz, 1H), 7.38 (s, 1H), 7.29−7.20 (m, 3H), 7.14 (d, J = 7.7 Hz, 1H), 6.64 (t, J = 6.1 Hz, 1H), 6.43 (s, 1H), 4.07 (s, 2H), 4.00 (t, J = 8.6 Hz, 2H), 3.76 (t, J = 5.5 Hz, 2H), 3.69−3.50 (m, 4H), 3.23 (s, 1H), 1.82 (p, J = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 166.2, 164.2, 160.9, 159.5, 158.0, 144.6, 141.8 (d, J = 7.5 Hz), 132.6, 131.0, 127.9, 121.8−121.6 (m), 119.8, 119.5, 118.8, 111.7−111.0 (m), 103.9, 59.9, 48.8, 43.8, 37.0, 32.1, 27.7. HRMS (ESI): (M + H)$^+$ calcd for $C_{24}H_{23}F_4N_4O_2$, 475.1752; found, 475.1749.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*-(4-hydroxybutyl)indoline-4-carboxamide (30d). Compound **30d** (30 mg, 61%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d* and MeOD) δ 8.66 (s, 1H), 8.52 (d, J = 8.1 Hz, 1H), 7.33 (s, 1H), 7.27−7.06 (m, 5H), 6.39 (s, 1H), 4.02 (s, 2H), 3.94 (t, J = 8.5 Hz, 2H), 3.61 (t, J = 5.8 Hz, 2H), 3.47 (t, J = 8.5 Hz, 2H), 3.38 (t, J = 6.2 Hz, 2H), 1.63 (dq, J = 12.9, 6.7 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ and MeOD) δ 168.5, 165.9, 164.1, 160.8, 159.5, 157.7, 144.2, 141.6 (d, J = 7.7 Hz), 132.3, 131.5, 127.7, 121.6, 120.3, 119.7, 119.4, 118.5, 111.4 (d, J = 3.8 Hz), 111.1 (d, J = 3.7 Hz), 104.0, 61.7, 48.8, 43.3, 39.6, 29.6, 27.4, 25.9. HRMS (ESI): (M + H)$^+$ calcd for $C_{25}H_{25}F_4N_4O_2$, 489.1908; found, 489.1904.

(1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-indolin-4-yl)(3-hydroxyazetidin-1-yl)methanone (30e). Compound **30e** (17 mg, 37%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.76 (d, J = 1.0 Hz, 1H), 8.56 (d, J = 8.2 Hz, 1H), 7.38 (s, 1H), 7.24 (d, J = 8.4 Hz, 3H), 7.00 (dd, J = 7.7, 1.0 Hz, 1H), 6.45−6.39 (m, 1H), 4.79−4.66 (m, 1H), 4.41 (s, 2H), 4.11−3.94 (m, 6H), 3.52−3.39 (m, 2H), 3.06 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 166.2, 164.2, 160.9, 159.5, 158.0, 144.5, 141.7 (d, J = 7.6 Hz), 132.0, 129.5, 127.5, 121.8−121.6 (m), 120.9, 119.6 (d, J = 21.7 Hz), 118.3, 111.3 (d, J = 28.0 Hz), 103.9, 62.2, 61.7, 58.2, 48.7, 43.7, 27.2. HRMS (ESI): (M + H)$^+$ calcd for $C_{24}H_{21}F_4N_4O_2$, 473.1595; found, 473.1593.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*-(2-fluoroethyl)indoline-4-carboxamide (30f). Compound **30f** (36 mg, 78%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.83−8.73 (m, 1H), 8.66 (d, J = 8.1 Hz, 1H), 7.39 (s, 1H), 7.33−7.15 (m, 4H), 6.45 (d, J = 9.4 Hz, 2H), 4.71 (t, J = 4.7 Hz, 1H), 4.55 (t, J = 4.7 Hz, 1H), 4.14−3.95 (m, 4H), 3.83 (q, J = 5.1 Hz, 1H), 3.74 (q, J = 5.1 Hz, 1H), 3.58 (t, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 166.3, 164.2, 160.9, 159.5, 158.0, 144.7, 141.8 (d, J = 7.6 Hz), 132.6, 130.8, 128.0, 121.7 (t, J = 3.5 Hz), 119.9, 119.8, 119.5, 118.9, 111.5 (d, J = 3.7 Hz), 111.2 (d, J = 3.8 Hz), 103.9, 82.8 (d, J = 166.7 Hz), 48.8, 43.8, 40.2 (d, J = 19.5 Hz), 27.7. ^{19}F NMR (282 MHz, chloroform-*d*) δ −62.7, −110.6 (t, J = 8.8 Hz), −224.2 (tt, J = 47.4, 28.3 Hz). HRMS (ESI): (M + H)$^+$ calcd for $C_{23}H_{20}F_5N_4O$, 473.1595; found, 473.1593.

1-(6-(3-Fluoro-5-(trifluoromethyl)benzyl)pyrimidin-4-yl)-*N*,*N*-bis(2-hydroxyethyl)indoline-4-carboxamide (30g). Compound **30g** (27 mg, 53%) was synthesized by a procedure similar to that used to prepare compound **12a** as a white solid. ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.71 (s, 1H), 8.45 (d, J = 8.2 Hz, 1H), 7.37 (s, 1H), 7.29−7.17 (m, 3H), 6.97 (d, J = 7.6 Hz, 1H), 6.40 (s, 1H), 4.10−3.57 (m, 12H), 3.44 (t, J = 5.0 Hz, 2H), 3.26 (t, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 166.2, 164.2, 160.9, 159.5, 158.0, 144.0, 141.7 (d, J = 7.5 Hz), 133.0, 129.7, 127.8, 122.0−121.5 (m), 120.2, 119.8, 119.5, 117.0, 111.3 (d, J = 24.4 Hz), 103.8, 61.1, 60.1, 52.9, 49.1, 48.5, 43.6, 26.1. HRMS (ESI): (M + H)$^+$ calcd for $C_{25}H_{25}F_4N_4O_3$, 505.1857; found, 505.1853.

Molecular Docking Protocol. The molecular docking study was performed using Schrödinger Small-Molecule Drug Discovery Suite (Schrödinger, LLC, New York, NY, 2020). The cocrystal structures of GPR52 and compound **2** (PDB code: 6LI0) were downloaded from the RCS PDB bank. The cocrystal structure was preprocessed and minimized with Schrödinger Protein Preparation Wizard using default settings. The grid center was chosen on the centroid of an existing ligand, and the size of the grid box was set to 30 Å on each side. The 3D structure of ligand **12c** was created using Schrödinger Maestro, and a low energy conformation was calculated using LigPrep. Docking was employed with Glide using the SP precision. Docked poses were incorporated into Schrödinger Maestro for visualization and analysis of binding site interactions.

***In Vitro* Pharmacology.** *Cell Culture and Plasmids.* Wildtype (WT) human embryonic kidney 293 (HEK293) cells were a generous gift from Dr. Asuka Inoue, Tohoku University.[31] The wildtype HEK293 cells were cultured in DMEM (Gibco, Carlsbad, CA) with 10% FBS (Omega Scientific, Tarzana, CA) and 10,000 U/mL penicillin−streptomycin (Gibco, Carlsbad, CA) in a humidified incubator at 37 °C in 5% CO_2. A GPR52 TANGO construct was purchased from Addgene (Watertown, MA). A stop codon was placed in the frame immediately after the receptor coding sequence to generate a human GPR52 WT coding construct. Point mutation of

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

GPR52 WT from GPR52 TANGO was made by polymerase chain reaction (PCR) using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol. Mutagenesis and sequencing primers were obtained from ThermoFisher (Pittsburgh, PA). The primers used to make the GPR52 WT point mutant were 5′−gctaacagctgttccatctaa-gataccggtggacgcacc−3′ (sense) and 5′−cgattgtcgacaaggtagattctatggc-cacctgcgtgg−3′ (antisense). Parental DNA in the reaction mixture was digested using restriction endonuclease DpnI (from diplococcus pneumoniae) at 37 °C for one hour. A digestion mixture (2 μL) was transformed into XL1-Blue supercompetent cells by heat shock at 42 °C for 45 s. The reaction was incubated in Super Optimal broth with a catabolite repression medium (Sigma-Aldrich) and then plated onto Luria−Bertani (LB) agar plates containing 100 μg/mL ampicillin. A single colony was selected for sequencing, and the point mutation was verified prior to use (Molecular Genomics Core, University of Texas Medical Branch, Galveston, TX).

Transfections. For the cAMP Glosensor assays, 6.5×10^5 wildtype HEK293 cells were plated in 6-well plates (Corning, Oneonta, NY) 24 h before transfection to achieve 70−80% confluency the following day. Each well of wildtype HEK293 cells was transiently transfected with 0.10 μg of wildtype human GPR52 plasmid cDNA and 1 μg of the 22F Glosensor plasmid (Promega, Madison, WI) using 10 μL of Lipofectamine 2000 (Invitrogen, Waltham, MA) in 1 mL of OptiMEM (Gibco, Carlsbad, CA) and 1 mL of growth media.

cAMP Glosensor Assay. Eighteen hours after transfection, wildtype HEK293 cells with GPR52 and Glosensor constructs (see Transfections above), cells were seeded at 60,000 cells/well in growth media into poly-L-lysine (Trevigen, Gaithersburg, MD) coated 96-well white clear-bottom cell culture plates (Greiner Bio-One, Monroe, NC). Four hours later, complete media was aspirated and replaced with 90 μL of a 1% Glosensor reagent (Promega, Madison, WI) in 1× Hanks' balanced salt solution (HBSS) and 20 mM HEPES. Cells were serum-starved for 2 h at room temperature in the dark. Test compounds were weighed and diluted to 2 mM in DMSO. Serial dilutions of each test compound were prepared at 10× final concentration and transferred to a 96-well source plate. Cells were incubated with 10 μL of 10× drug solutions for 15 min. LCPS were recorded on a Microbeta2 Microplate counter (PerkinElmer, Waltham, MA). Data from at least three independent experiments (n = 3 to 21) conducted in technical triplicate are presented as percentage of compound **4** response. All *in vitro* pharmacological data were analyzed using GraphPad Prism 7.05 software (La Jolla, CA). Data from cAMP ligand dose response assays are presented as the half maximum (EC_{50}, nM) and maximum effect (E_{max}) (means ± SEM) as computed by GraphPad using a four-parameter nonlinear regression curve-fitting algorithm. All E_{max} ligand responses were scaled relative to 3 μM compound **4** in cells treated for each assay (3 μM compound **4** response set to 100%).

In Vivo PK and Brain Permeability Studies. Male Sprague Dawley rats (n = 3 per treatment group; Beijing Vital River Laboratory, Animal Technology Co., Ltd., Beijing, China) weighing 200−250 g at the beginning of the experiment were housed three per cage in a pathogen-free, temperature-controlled (20−26 °C), and humidity-controlled (40−70%) environment with a 12 h light−dark cycle and *ad libitum* access to food and filtered water. Rats were randomly assigned to treatment groups. A vehicle [10% dimethyl sulfoxide (DMSO) and 90% 2-hydroxypropyl-β-cyclodextrin (HP-β-CD); Cyclodextrin Technologies Development, Inc., High Springs, FL, USA] or compound **12c** dissolved in the vehicle was administered to rats IV at 10 mg/kg or PO at 20 mg/kg. The rat was restrained manually at the designated time points (0.08, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 24 h post-dosing for IV; 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 24 h post-dosing for PO). Blood (yielding ∼50 μL of plasma) samples (100 μL) are taken from the animal's jugular vein and placed into micro K_2EDTA tubes. Blood samples were placed on ice and centrifuged at 8000 rpm for 5 min at 4 °C to generate plasma samples within 0.5 h of collection. Brain samples were collected at 0.25 and 1 h post-dosing. All samples were stored at −20 °C. The concentration of **12c** in each sample was analyzed by Sundia MediTech Co., Ltd. The study and the related standard operating procedures were reviewed and approved by the Sundia Institutional Animal Care and Use Committee. The Sundia animal facility is approved with yearly inspection by the Shanghai Laboratory Animal Management Committee. All treatment assignments were blinded to investigators who performed PK assays and end point statistical analyses.

The PK parameters of compound **12c** were calculated according to a non-compartmental model using WinNonlin (Pharsight Corporation, ver 5.3, Mountain View, CA, USA). The peak concentration (C_{max}) was directly obtained by visual inspection of the plasma concentration−time profile. The elimination rate constant (λ) was obtained by the least-squares fitted terminal log-linear portion of the slope of the plasma concentration−time profile. The elimination half-life ($t_{1/2}$) was evaluated according to $0.693/\lambda$. The area under the plasma concentration−time curve from 0 to time t (AUC_{0-t}) was evaluated using the linear trapezoidal rule and further extrapolated to infinity ($AUC_{0-\infty}$) according to the following equation: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/\lambda$. The pharmacokinetic parameters were presented as mean ± SEM.

***In Vivo* Pharmacology of Compound 12c.** *Animals.* Naïve male C57/BL6 mice (n = 60; Jackson Laboratory, Bar Harbor, ME) weighing between 24 and 31 g (∼60 days of age) at the beginning of the experiment were housed four/cage in a temperature- (21−23 °C) and humidity-controlled (40−50%) environment on a 12 h light/dark cycle (lights on at 0600). Mice were provided *ad libitum* access to chow and water for 14−20 days prior to experiments, which were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011) and with the approval of the Institutional Animal Care and Use Committee at the University of Texas Medical Branch.

Drugs. Compound **12c** was dissolved in 0.9% saline containing 20% HP-β-CD with a final pH of the solution adjusted to 7.4. Dextroamphetamine (Sigma-Aldrich, St Louis, MO, USA) was prepared in 0.9% saline. Compounds were then administered IP at 2 mL/1 kg body weight.

Locomotor Activity Assessment. A modified open field activity system (San Diego Instruments, San Diego, California, U.S.A.) was employed to quantify activity under low-light conditions.[32−34] Clear Plexiglas chambers (40 × 40 × 40 cm) were surrounded by a 4 × 4 photobeam matrix positioned ∼1 cm above the chamber floor. Horizontal activity was quantified as the sum of photobeam breaks that occurred within the inner central 16 × 16 cm area and in the surrounding outer 12 × 12 cm perimeter of the activity monitor.

A between-subjects design was employed to study the efficacy of compound **12c** to impact horizontal activity. Mice were handled three times/week for two weeks prior to the study and were habituated to the motor activity monitors 1 h/day for three days. Mice were transported in their home cages and allowed to acclimate to the testing room for 1 h prior to evaluation. On the day of the test, mice were weighed and randomized into five treatment groups for compound **12c** administration (n = 12/dose). One animal was excluded as an outlier (i.e., horizontal activity two standard deviations from the mean) with n = 11 for 1 mg/kg compound **12c**). Following IP administration of the vehicle or compound **12c** (0.3, 1, 3, and 10 mg/kg), automated activity monitoring began immediately and continued for 30 min. Mice were then injected with AMPH (3 mg/kg IP), and activity was monitored for the next 90 min. All testings occurred between 0800 and 1700 h.

Statistical Analyses. Locomotor activities are presented as mean horizontal activity (mean ± SEM). A one-way ANOVA for a between-subjects design was employed to analyze horizontal activity. *A priori* comparisons were defined prior to the start of experimentation and conducted by Dunnett's procedure,[35] with an experiment-wise error rate set at α = 0.05. Investigators who performed compound administration were blinded to treatment assignments and endpoint statistical analyses.

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01498.

Figures S1 and S2, HPLC analysis spectra of representative compounds, and ^{1}H and ^{13}C NMR spectra of all new compounds (PDF)

Molecular formula strings and data (CSV)

Docking compound **12c** with GPR52 (PDB)

■ AUTHOR INFORMATION

Corresponding Authors

John A. Allen − *Chemical Biology Program, Department of Pharmacology and Toxicology and Center for Addiction Research, University of Texas Medical Branch, Galveston, Texas 77555, United States*; Phone: (409) 772-9621; Email: joaallen@utmb.edu; Fax: 409-747-7050

Jia Zhou − *Chemical Biology Program, Department of Pharmacology and Toxicology and Center for Addiction Research, University of Texas Medical Branch, Galveston, Texas 77555, United States*; orcid.org/0000-0002-2811-1090; Phone: (409) 772-9748; Email: jizhou@utmb.edu; Fax: (409) 772-9648

Authors

Pingyuan Wang − *Chemical Biology Program, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555, United States*

Daniel E. Felsing − *Center for Addiction Research, University of Texas Medical Branch, Galveston, Texas 77555, United States*

Haiying Chen − *Chemical Biology Program, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555, United States*

Sonja J. Stutz − *Center for Addiction Research, University of Texas Medical Branch, Galveston, Texas 77555, United States*

Ryan E. Murphy − *Center for Addiction Research, University of Texas Medical Branch, Galveston, Texas 77555, United States*

Kathryn A. Cunningham − *Chemical Biology Program, Department of Pharmacology and Toxicology and Center for Addiction Research, University of Texas Medical Branch, Galveston, Texas 77555, United States*; orcid.org/0000-0002-4257-1739

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c01498

Author Contributions

[§]P.W. and D.E.F contributed equally and should be considered as cofirst authors.

Author Contributions

[‖]J.A.A and J.Z. are equal contributors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants T32 DA 007287 and U18 DA052543 from the National Institutes of Health, the PhRMA foundation, and the Center for Addiction Research at the University of Texas Medical Branch (UTMB). J.Z. is also partially supported by the John D. Stobo, M. D., Distinguished Chair Endowment Fund. The authors would like to acknowledge Robert Fox for *in vivo* assay assistance and the preparation of amphetamine solutions for *in vivo* injections. The authors would also like to acknowledge Dr. Asuka Inoue, Tohoku University, for the generous gift of the HEK cell line, Dr. Lawrence C. Sowers at the UTMB Department of Pharmacology and Toxicology as well as Dr. Tianzhi Wang at the NMR core facility of UTMB for the NMR spectroscopy assistance, and Dr. Xuemei Luo at the UTMB Mass Spectrometry Core with the funding support from the University of Texas System Proteomics Network for the HRMS analysis.

■ ABBREVIATIONS USED

GPCRs, G-protein-coupled receptors; FDA, U.S. Food and Drug Administration; CNS, central nervous system; NAc, nucleus accumbens; MSNs, medium spiny neurons; cAMP, cyclic adenosine monophosphate; NMDA, *N*-methyl-D-aspartate; SUD, substance use disorder; ECL2, extracellular loop 2; EC_{50}, half maximal effective concentration; HTS, high-throughput screening; TM, transmembrane helix; SAR, structure−activity relationship; PK, pharmacokinetic; DMF, *N*,*N*-dimethylformamide; EDCI, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; XantPhos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; *m*CPBA, *meta*-chloroperoxybenzoic acid; DAST, diethylaminosulfur trifluoride; DMSO, dimethyl sulfoxide; NIMH, National Institute of Mental Health; PDSP, psychoactive drug screening program; hERG, human ether-a-go-go-related gene; PO, oral; IV, intravenous; AMPH, amphetamine; WT, wildtype; HEK293, human embryonic kidney 293; PCR, polymerase chain reaction; HBSS, Hanks' balanced salt solution; IP, intraperitoneal

■ REFERENCES

(1) Allen, J. A.; Roth, B. L. Strategies to discover unexpected targets for drugs active at G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* **2011**, *51*, 117−144.

(2) Wold, E. A.; Chen, J.; Cunningham, K. A.; Zhou, J. Allosteric modulation of class A GPCRs: targets, agents, and emerging concepts. *J. Med. Chem.* **2019**, *62*, 88−127.

(3) Zhou, J.; Wild, C. GPCR drug discovery: emerging targets, novel approaches and future trends. *Curr. Top. Med. Chem.* **2019**, *19*, 1363−1364.

(4) Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug Discovery* **2006**, *5*, 993−996.

(5) Lagerström, M. C.; Schiöth, H. B. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discovery* **2008**, *7*, 339−357.

(6) Hauser, A. S.; Attwood, M. M.; Rask-Andersen, M.; Schiöth, H. B.; Gloriam, D. E. Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discovery* **2017**, *16*, 829−842.

(7) Roth, B. L.; Irwin, J. J.; Shoichet, B. K. Discovery of new GPCR ligands to illuminate new biology. *Nat. Chem. Biol.* **2017**, *13*, 1143−1151.

(8) Ye, N.; Li, B.; Mao, Q.; Wold, E. A.; Tian, S.; Allen, J. A.; Zhou, J. Orphan receptor GPR88 as an emerging neurotherapeutic target. *ACS Chem. Neurosci.* **2019**, *10*, 190−200.

(9) Sawzdargo, M.; Nguyen, T.; Lee, D. K.; Lynch, K. R.; Cheng, R.; Heng, H. H. Q.; George, S. R.; O'Dowd, B. F. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, ΨGPR53 and GPR55: GPR55 is extensively expressed in human brain. *Mol. Brain Res.* **1999**, *64*, 193−198.

(10) Komatsu, H.; Maruyama, M.; Yao, S.; Shinohara, T.; Sakuma, K.; Imaichi, S.; Chikatsu, T.; Kuniyeda, K.; Siu, F. K.; Peng, L. S.; Zhuo, K.; Mun, L. S.; Han, T. M.; Matsumoto, Y.; Hashimoto, T.; Miyajima, N.; Itoh, Y.; Ogi, K.; Habata, Y.; Mori, M. Anatomical transcriptome of G protein-coupled receptors leads to the identification of a novel therapeutic candidate GPR52 for psychiatric disorders. *PLoS One* **2014**, *9*, No. e90134.
(11) Yang, R. Y.; Quan, J.; Sodaei, R.; Aguet, F.; Segrè, A. V.; Allen, J. A.; Lanz, T. A.; Reinhart, V.; Crawford, M.; Hasson, S.; Ardlie, K. G.; Guigö, R.; Xi, H. S. A systematic survey of human tissue-specific gene expression and splicing reveals new opportunities for therapeutic target identification and evaluation. *bioRxiv* **2018**, 311563.
(12) Lin, X.; Li, M.; Wang, N.; Wu, Y.; Luo, Z.; Guo, S.; Han, G. W.; Li, S.; Yue, Y.; Wei, X.; Xie, X.; Chen, Y.; Zhao, S.; Wu, J.; Lei, M.; Xu, F. Structural basis of ligand recognition and self-activation of orphan GPR52. *Nature* **2020**, *579*, 152−157.
(13) Martin, A. L.; Steurer, M. A.; Aronstam, R. S. Constitutive activity among orphan class-A G protein coupled receptors. *PLoS One* **2015**, *10*, No. e0138463.
(14) Setoh, M.; Ishii, N.; Kono, M.; Miyanohana, Y.; Shiraishi, E.; Harasawa, T.; Ota, H.; Odani, T.; Kanzaki, N.; Aoyama, K.; Hamada, T.; Kori, M. Discovery of the first potent and orally available agonist of the orphan G-protein-coupled receptor 52. *J. Med. Chem.* **2014**, *57*, 5226−5237.
(15) Nishiyama, K.; Suzuki, H.; Harasawa, T.; Suzuki, N.; Kurimoto, E.; Kawai, T.; Maruyama, M.; Komatsu, H.; Sakuma, K.; Shimizu, Y.; Shimojo, M. FTBMT, a novel and selective GPR52 agonist, demonstrates antipsychotic-like and procognitive effects in rodents, revealing a potential therapeutic agent for schizophrenia. *J. Pharmacol. Exp. Ther.* **2017**, *363*, 253−264.
(16) Xiong, Y. G. A. J.; Korman, H.; Lehmann, J.; Ren, A. S.; Semple, G. 1-Heteroaryl-indoline-4-carboxamines as modulators of GPR52 usedul for the treatment or prevention of disorders related thereto. WO2016176571 A1, Nov 3, 2016.
(17) Tokumaru, K.; Ito, Y.; Nomura, I.; Nakahata, T.; Shimizu, Y.; Kurimoto, E.; Aoyama, K.; Aso, K. Design, synthesis, and pharmacological evaluation of 4-azolyl-benzamide derivatives as novel GPR52 agonists. *Bioorg. Med. Chem.* **2017**, *25*, 3098−3115.
(18) Nakahata, T.; Tokumaru, K.; Ito, Y.; Ishii, N.; Setoh, M.; Shimizu, Y.; Harasawa, T.; Aoyama, K.; Hamada, T.; Kori, M.; Aso, K. Design and synthesis of 1-(1-benzothiophen-7-yl)-1H-pyrazole, a novel series of G protein-coupled receptor 52 (GPR52) agonists. *Bioorg. Med. Chem.* **2018**, *26*, 1598−1608.
(19) Meanwell, N. A. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* **2011**, *54*, 2529−2591.
(20) Meanwell, N. A. The influence of bioisosteres in drug design: tactical applications to address developability problems. *Tactics Contemp. Drug Des.* **2015**, *9*, 283−382.
(21) Pennington, L. D.; Moustakas, D. T. The necessary nitrogen atom: a versatile high-impact design element for multiparameter optimization. *J. Med. Chem.* **2017**, *60*, 3552−3579.
(22) Cesta, D. J.; Somersan Karakaya, S. S.; Saito, K.; Javidnia, P. E.; Roberts, J.; Ling, Y.; Gold, B. S.; Lopez Quezada, L.; Zhang, D.; Abramyan, T. M.; Kireev, D. B.; Aubé, J.; Scarry, S. M. Synthesis and antibacterial evaluation of cephalosporin isosteres. *Asian J. Org. Chem.* **2019**, *8*, 1053−1057.
(23) Sengmany, S.; Lebre, J.; Le Gall, E.; Léonel, E. Selective mono-amination of dichlorodiazines. *Tetrahedron* **2015**, *71*, 4859−4867.
(24) Loksha, Y. M. Novel synthetic route for 5-substituted 6-arylmethylluracils from 2,4,6-trichloropyrimidines. *J. Heterocycl. Chem.* **2009**, *46*, 1296−1301.
(25) Gray, D. L.; Allen, J. A.; Mente, S.; O'Connor, R. E.; DeMarco, G. J.; Efremov, I.; Tierney, P.; Volfson, D.; Davoren, J.; Guilmette, E.; Salafia, M.; Kozak, R.; Ehlers, M. D. Impaired β-arrestin recruitment and reduced desensitization by non-catechol agonists of the D1 dopamine receptor. *Nat. Commun.* **2018**, *9*, 674.
(26) Besnard, J.; Ruda, G. F.; Setola, V.; Abecassis, K.; Rodriguiz, R. M.; Huang, X.-P.; Norval, S.; Sassano, M. F.; Shin, A. I.; Webster, L. A.; Simeons, F. R. C.; Stojanovski, L.; Prat, A.; Seidah, N. G.; Constam, D. B.; Bickerton, G. R.; Read, K. D.; Wetsel, W. C.; Gilbert, I. H.; Roth, B. L.; Hopkins, A. L. Automated design of ligands to polypharmacological profiles. *Nature* **2012**, *492*, 215−220.
(27) Kalyaanamoorthy, S.; Barakat, K. H. Development of safe drugs: the hERG challenge. *Med. Res. Rev.* **2018**, *38*, 525−555.
(28) Jones, C. A.; Watson, D. J. G.; Fone, K. C. F. Animal models of schizophrenia. *Br. J. Pharmacol.* **2011**, *164*, 1162−1194.
(29) Dichter, G. S.; Damiano, C. A.; Allen, J. A. Reward circuitry dysfunction in psychiatric and neurodevelopmental disorders and genetic syndromes: animal models and clinical findings. *J. Neurodev. Disord.* **2012**, *4*, 19.
(30) Komatsu, H. Novel therapeutic GPCRs for psychiatric disorders. *Int. J. Mol. Sci.* **2015**, *16*, 14109−14121.
(31) Grundmann, M.; Merten, N.; Malfacini, D.; Inoue, A.; Preis, P.; Simon, K.; Rüttiger, N.; Ziegler, N.; Benkel, T.; Schmitt, N. K.; Ishida, S.; Müller, I.; Reher, R.; Kawakami, K.; Inoue, A.; Rick, U.; Kühl, T.; Imhof, D.; Aoki, J.; König, G. M.; Hoffmann, C.; Gomeza, J.; Wess, J.; Kostenis, E. Lack of beta-arrestin signaling in the absence of active G proteins. *Nat. Commun.* **2018**, *9*, 341.
(32) Simmler, L. D.; Anacker, A. M. J.; Levin, M. H.; Vaswani, N. M.; Gresch, P. J.; Nackenoff, A. G.; Anastasio, N. C.; Stutz, S. J.; Cunningham, K. A.; Wang, J.; Zhang, B.; Henry, L. K.; Stewart, A.; Veenstra-VanderWeele, J.; Blakely, R. D. Blockade of the 5-HT transporter contributes to the behavioural, neuronal and molecular effects of cocaine. *Br. J. Pharmacol.* **2017**, *174*, 2716−2738.
(33) Cortez, I.; Bulavin, D. V.; Wu, P.; McGrath, E. L.; Cunningham, K. A.; Wakamiya, M.; Papaconstantinou, J.; Dineley, K. T. Aged dominant negative p38α MAPK mice are resistant to age-dependent decline in adult-neurogenesis and context discrimination fear conditioning. *Behav. Brain Res.* **2017**, *322*, 212−222.
(34) Cunningham, K. A.; Anastasio, N. C.; Fox, R. G.; Stutz, S. J.; Bubar, M. J.; Swinford, S. E.; Watson, C. S.; Gilbertson, S. R.; Rice, K. C.; Rosenzweig-Lipson, S.; Moeller, F. G. Synergism between a serotonin 5-HT_{2A} receptor (5-HT_{2A}R) antagonist and 5-HT_{2C}R agonist suggests new pharmacotherapeutics for cocaine addiction. *ACS Chem. Neurosci.* **2013**, *4*, 110−121.
(35) Keppel, G.; Wickens, T. D. *Design and analysis: a researcher's handbook*; Pearson Prentice Hall: 2004.

https://dx.doi.org/10.1021/acs.jmedchem.0c01498
J. Med. Chem. XXXX, XXX, XXX−XXX