Journal of Medicinal Chemistry

pubs.acs.org/jmc

Article

Design, Synthesis, and Biological Evaluation of Linear Aliphatic Amine-Linked Triaryl Derivatives as Potent Small-Molecule Inhibitors of the Programmed Cell Death-1/Programmed Cell Death-Ligand 1 Interaction with Promising Antitumor Effects In Vivo

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Cite This: https://dx.doi.org/10.1021/acs.jmedchem.0c01329

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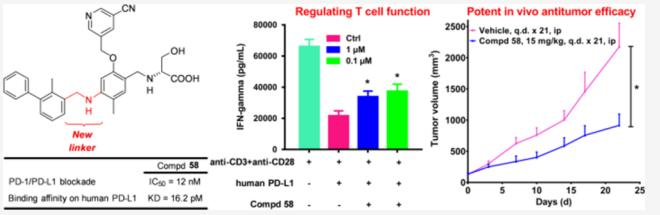
ABSTRACT: A series of novel linear aliphatic amine-linked triaryl derivatives as inhibitors of PD-1/PD-L1 were designed, synthesized, and evaluated in vitro and in vivo. In this chemical series, compound **58** showed the most potent inhibitory activity and binding affinity with hPD-L1, with an IC_{50} value of 12 nM and a KD value of 16.2 pM, showing a binding potency approximately 2000-fold that of hPD-1. Compound **58** could bind with hPD-L1 on the cellular surface and competitively block the interaction of hPD-1 with hPD-L1. In a T cell function assay, **58** restored the T cell function, leading to increased IFN-γ secretion. Moreover, in a humanized mouse model, compound **58** significantly inhibited tumor growth without obvious toxicity and showed moderate PK properties after intravenous injection. These results indicated that **58** is a promising lead for further development of small-molecule PD-1/PD-L1 inhibitors for cancer therapy.



INTRODUCTION

Cancer immunotherapy activates and enhances pre-existing anticancer immune responses of the host immune system to eliminate cancer cells.[1−3] Undoubtedly, the most remarkable progress in this field is the development of programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) checkpoint inhibitors. PD-1 is expressed on the surface of immune effector cells, including T cells, B cells, natural killer T cells, monocytes, and dendritic cells. PD-L1 is constitutively expressed on immune cells and the cells of various tissues, including the heart, lung, liver, pancreatic islet, vascular endothelium, atrocities, etc.[4,5] Under normal physiological conditions, the interaction of PD-1 and PD-L1 suppresses the proliferation, inflammatory cytokine secretion, such as that of IL-2 and interferon-γ (IFN-γ), and cytotoxic activity of T cells and increases their susceptibility to apoptosis, thereby maintaining self-tolerance and protecting tissues from unnecessary damage during immune responses.[6−8] However, tumor cells exploit this mechanism to evade immune surveillance and facilitate their own survival by interaction between overexpressed PD-L1 on their surface and PD-1 on T cells.[1,9]

Several clinical trials of humanized monoclonal antibodies (mAbs) targeting PD-1/PD-L1 have exhibited dramatic antitumor efficacy and long-term remission in patients.[9−11] Blocking the PD-1/PD-L1 interaction has been one of the most promising strategies for cancer treatment. To date, six FDA-approved mAbs targeting PD-1 or PD-L1 (anti-PD-1: nivolumab, pembrolizumab, and cemiplimab; anti-PD-L1: atezolizumab, avelumab, and durvalumab) are clinically available.[7,12] In addition, numerous mAbs have entered clinical trials worldwide.[13,14]

Despite the good clinical efficacy, durable responses, and prolonged survival achieved with mAbs, their intrinsic defects limit their widespread clinical application. In comparison with mAbs, small-molecule inhibitors have more appropriate pharmacokinetic (PK) properties, stability, and oral bioavailability; broader distribution; lower costs of production, transportation, and storage; enhanced tumor and tissue penetration; relatively low immunogenicity; and easier reversal of immune-related adverse events due to their relatively shorter half-lives, which make them potential alternatives or complementary drugs to regulate the PD-1/PD-L1 pathway.[15−23] For these reasons, the discovery of small-molecule inhibitors has become an attractive strategy for inhibiting the PD-1/PD-L1 interaction, but their development is far behind that of mAbs.

Received: August 6, 2020

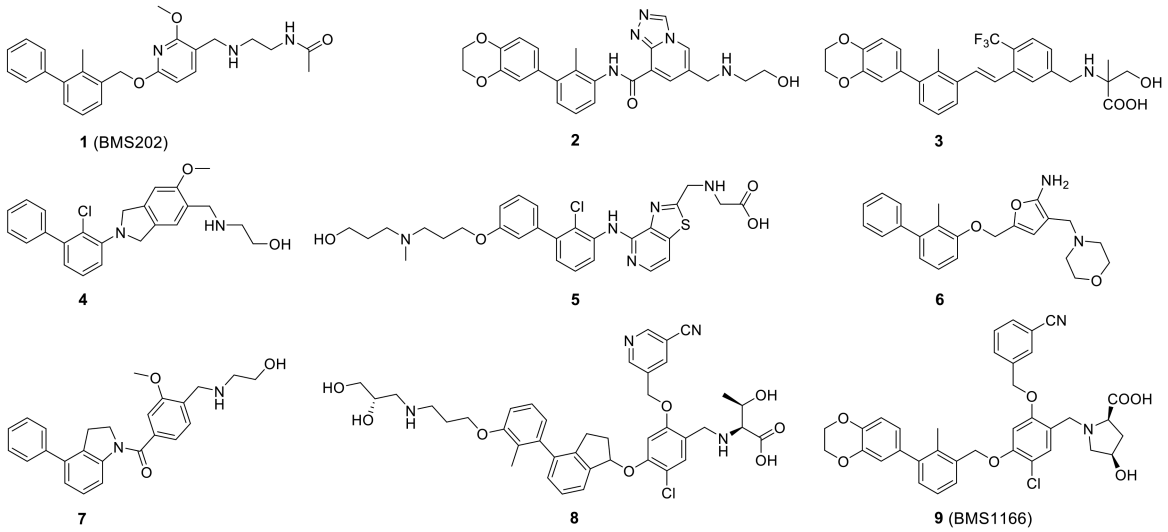


Figure 1. Chemical structures of reported small-molecule inhibitors of PD-1/PD-L1.

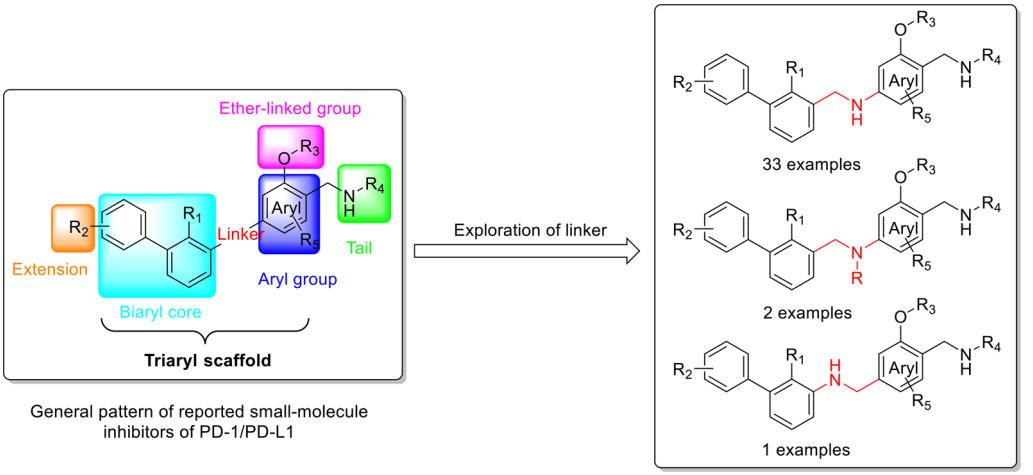


Figure 2. Design strategy of compounds.

To date, the majority of reported small-molecule inhibitors targeting the PD-1/PD-L1 pathway remain in the preclinical stage, and only CA-170 and IMMH-010 have entered phase I clinical trials. Compounds with triaryl scaffolds are the predominant class of small-molecule inhibitors thus far. The first small-molecule inhibitors of PD-1/PD-L1 with triaryl scaffolds, such as BMS202 and BMS1166, were disclosed by researchers from Bristol Myers Squibb (BMS) and are shown in Figure 1. Most of these compounds showed high inhibitory potency of the PD-1/PD-L1 interaction, with IC_{50} values in the nanomolar range.[24−26] Further investigation revealed that the BMS compounds bound to the PD-1 binding site of PD-L1 and induced dimerization of PD-L1, further occluding the PD-1 binding site of PD-L1.[27−29] Inspired by this remarkable progress, a series of triaryl scaffold derivatives were developed by other scientists (Figure 1). These derivatives also showed potential inhibitory activity in biochemical assays.[30−36] Despite the good biochemical activity of triaryl scaffold derivatives, no further investigation data in vitro and in vivo were disclosed to exhibit their potential antitumor and druggability properties.

To date, no small-molecule inhibitors have been clinically approved, nor have their antitumor effects been validated in clinical trials. The discovery and development of small-molecule PD-1/PD-L1 inhibitors with novel chemo-types, relatively high potency, appropriate PK properties, and optimized therapeutic effects are still needed. Although it is a challenge to discover a novel inhibitor due to the large, flat, and hydrophobic interface of PD-1/PD-L1,[37] further investigation, including modification and structure−activity relationship (SAR) studies, of reported inhibitors will lead to a new generation of highly potent inhibitors and accelerate the development of small-molecule inhibitors targeting PD-1/PD-L1.

In this paper, we designed and synthesized a series of novel triaryl derivatives with linear aliphatic amine linkers as small-molecule inhibitors targeting the PD-1/PD-L1 interaction; investigated their preliminary SARs; and evaluated their biochemical activities, binding specificity, affinity, binding and blocking ability at the cellular level, and effects on T cell function regulation using reasonable evaluation models in vitro. Then, we utilized an authentic pharmacodynamic model to evaluate the antitumor effect of the compounds in vivo. The results of biological evaluation in vitro and in vivo indicated that compound **58** was the most promising small-molecule inhibitor of PD-1/PD-L1 of these compounds, which remarkably inhibited tumor growth in a humanized mouse

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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model by inhibiting the PD-1/PD-L1 interaction, leading to restoration of antitumor immunity.

RESULTS AND DISCUSSION

Drug Design and Chemistry. As shown in Figure 2, reported small-molecule inhibitors with triaryl scaffolds shared structural features with the BMS compounds. According to the binding modes of BMS compounds with PD-L1,[27−29] with representative binding modes shown in Figure 3, the SARs of these reported small-molecule inhibitors with triaryl scaffolds were analyzed. The aryl group and biaryl core are linked together via a linker, which makes up the triaryl scaffold of the inhibitors. The biaryl core provides a significant contribution to inhibitor binding though hydrophobic interactions, such as $\pi-\pi$, π−alkyl, and $\pi-\sigma$ interactions. The aryl group generates a strong $\pi-\pi$ stacking with Tyr56 of the PD-L1 protein, which can tolerate a wide variety of aromatic heterocycles, including fused heterocycles. The ether-linked group is not essential, but its presence, especially that of a cyano-substituted aryl group, increases the binding potency. The extension is usually linked to the biaryl core via an ether bond, which is not necessary for inhibitor binding but is helpful for improving PK properties, especially regarding hydrophilic substituents. The tail is exposed to the solvent region and forms key hydrogen bond interactions with PD-L1, which is also required for high potency.

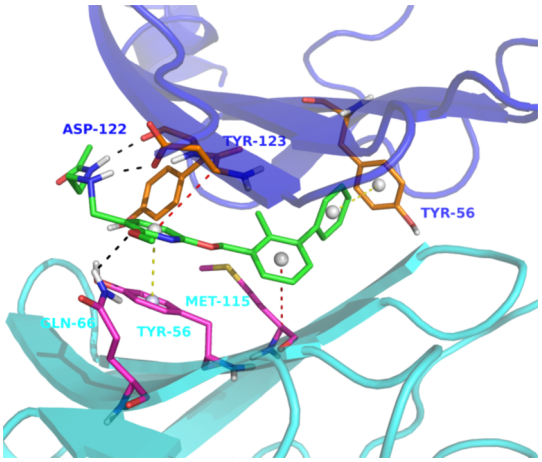
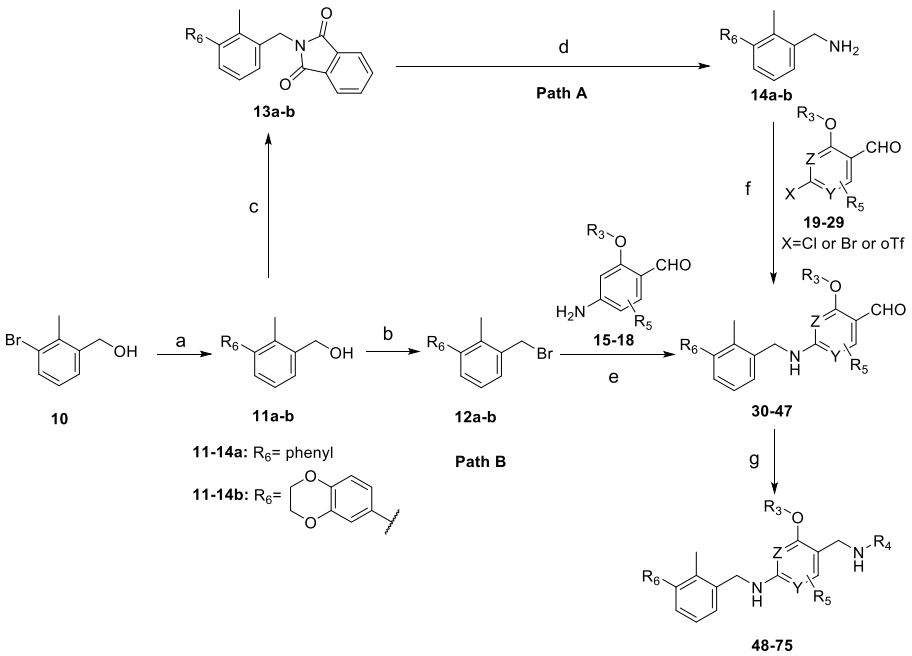


Figure 3. Representative binding mode of BMS compounds with PD-L1. BMS202 (green) is located in the hydrophobic cleft formed by dimeric PD-L1 (the PDB code for dimeric PD-L1 protein is 5J89).

On the basis of this SAR information, the exploration of linkers is likely a feasible way to develop novel inhibitors and further investigate the SARs of small-molecule inhibitors with triaryl scaffolds. Compared to other reported linkers, aliphatic amines are significantly different in geometry, and their introduction will generate inhibitors with different molecular conformations. Aliphatic amines have hydrogen bond donors and acceptors, which probably form undiscovered interactions with the Ala121 residue of PD-L1. In addition, the hydrophilicity of aliphatic amines is probably advantageous in terms of providing drug-like properties. On the basis of these considerations, three aliphatic amine linkers were designed to develop novel small-molecule inhibitors, as shown in Figure 2.

The syntheses of **48**−**75** are outlined in Scheme 1. The Suzuki−Miyaura coupling of **10** with phenylboronic acid or (2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)boronic acid yielded **11a** or **11b**, respectively. Paths A and B were designed to

Scheme 1. Syntheses of Compounds 48−75[a]



[a]Reagents and conditions: (a) $R_6B(OH)_2$, $PdCl_2dppf$, CsOAc, argon, THF, reflux, 23−24 h, and 98%. (b) PBr_3, DCM, 0 °C, 20 min, and 66−86%. (c) *o*-Phthalimide, Ph_3P, DEAD, argon, anhydrous THF, 0 °C−rt, 24 h, and 92−97%. (d) $H_4N_2{\cdot}H_2O$, EtOH, reflux, 3−6 h, and 96−99%. (e) K_2CO_3, MeCN/DMF, 60 °C−reflux, 10−18 h or 35 min (microwave), and 20−57%. (f) $Pd(OAc)_2$, BINAP or s-phos, Cs_2CO_3, argon, dioxane, 85 °C−reflux, 20−24 h, and 16−78%. (g) (1) R_4-NH_2, anhydrous DMF/MeOH/DCE, AcOH, rt, and 12−24 h; (2) $NaBH(OAc)_3$, 12−24 h, and 8−79%.

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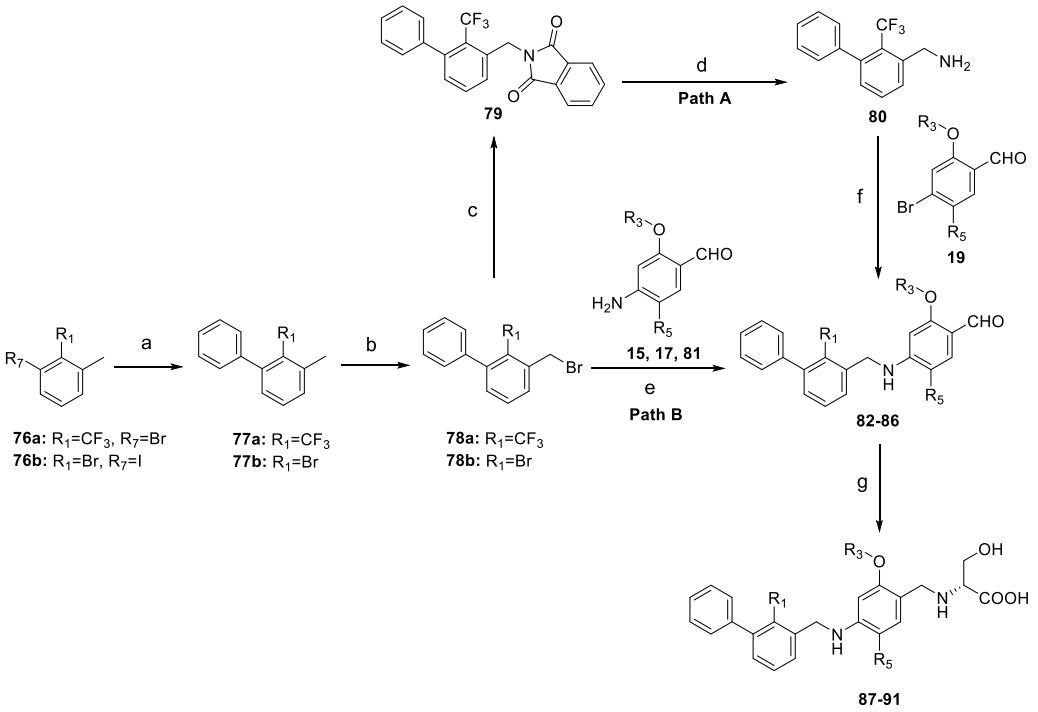
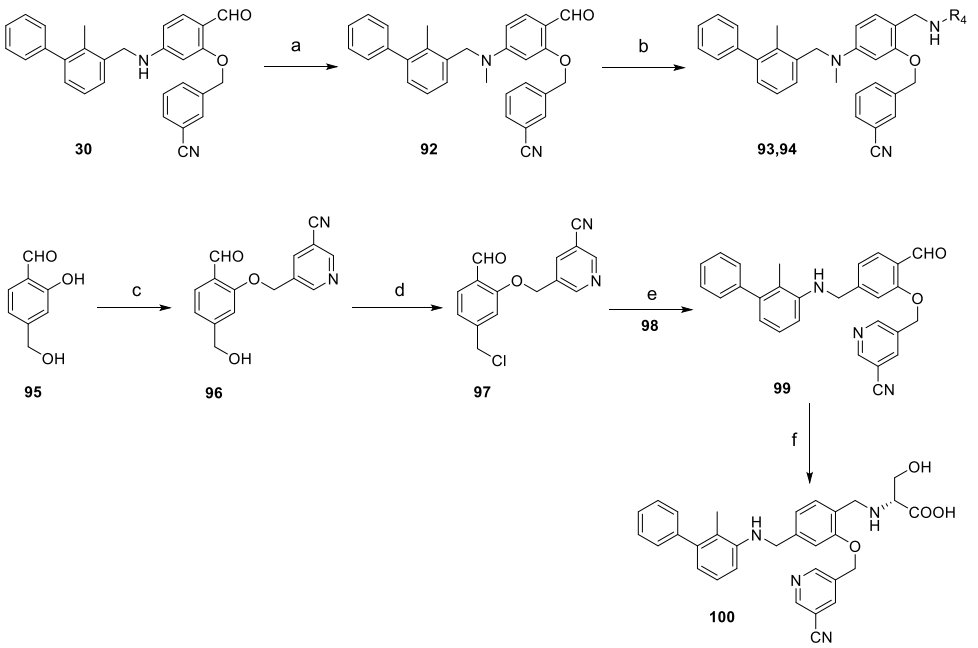
Scheme 2. Syntheses of Compounds 87−91[a]



[a]Reagents and conditions: (a) $PhB(OH)_2$, $PdCl_2dppf$, CsOAc, argon, THF, reflux, 36 h, and 52−95%. (b) NBS, BPO, CCl_4, reflux, 10−11 h, and 62−65%. (c) Phthalimide potassium salt, DMF, 80 °C, 1.5 h, and 60% (two steps from **77a**). (d) $H_4N_2·H_2O$, EtOH, reflux, 3 h, and 98%. (e) K_2CO_3, MeCN/DMF, 60 °C−reflux, 7.5−11 h, and 12−25%. (f) $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane, argon, reflux, 16 h, and 67%. (g) (1) D-serine, anhydrous DMF/MeOH, AcOH, 35 °C, and 24 h; (2) $NaBH(OAc)_3$, 24 h, and 22−70%.

Scheme 3. Syntheses of Compounds 93, 94, and 100[a]



[a]Reagents and conditions: (a) $PhSO_2OMe$, KOH, THF, 35 °C, 40 h, and 63%. (b) (1) R_4−NH_2, anhydrous DMF/DCE, AcOH, rt, and 24 h; (2) $NaBH(OAc)_3$, 12−14 h, and 38−60%. (c) 5-(Chloromethyl)nicotinonitrile, K_2CO_3, MeCN, 60 °C, 10 h, and 84%. (d) MsCl, pyridine, anhydrous DMF/DCM, 0 °C−rt, 24 h, and 63%. (e) **98**, K_2CO_3, MeCN/DMF, 30 °C, 24 h, and 31%. (f) (1) D-serine, anhydrous DMF/MeOH, AcOH, 35 °C, and 24 h; (2) $NaBH(OAc)_3$, 24 h, and 23%.

synthesize the key intermediates **30**−**47** using aryl halides **19**−**29** and aryl amines **15**−**18**, respectively. Intermediates **15**−**29** were purchased or synthesized as described in the Experimental Section. As described in path B, **12a-b** were prepared from **11a-b** using phosphorus tribromide, and then, K_2CO_3-promoted N-alkylation of **12a-b** and **15**−**18** was

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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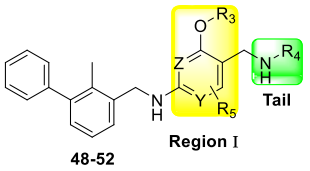
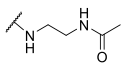
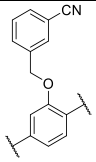
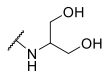
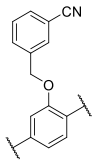
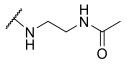
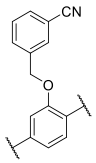
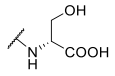
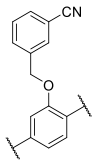
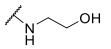
Table 1. Inhibitory Activities of Compounds 48−52 toward the PD-1/PD-L1 Interaction



48-52

Compound	Region I	Tail	IC_{50} (μM)[a]	Compound	Region I	Tail	IC_{50} (μM)[a]
48			4.243 ± 0.339	51			2.599 ± 0.244
49			4.942 ± 0.270	52			1.155 ± 0.091
50			3.673 ± 0.376	BMS202[b]	/	/	0.175 ± 0.014

[a]Data are displayed as averages of triplicate assays ± SD. [b]BMS202 reference value: IC_{50} = 18 nM.

performed to obtain the key intermediates **30**−**34**. As described in path A, the syntheses of **14a-b** employed the Mitsunobu reaction of **11a-b** with *o*-phthalimide followed by a hydrazinolysis reaction. The subsequent Buchwald−Hartwig cross-coupling reaction of **14a-b** and **19**−**29** provided the key intermediates **35**−**47**. The target molecules **48**−**75** were generated by reductive amination using $NaBH(OAc)_3$ and the appropriate amines.

The syntheses of **87**−**91** are shown in Scheme 2. Compounds **78a-b** were transformed from **76a-b** and phenylboronic acid using the Suzuki−Miyaura coupling reaction followed by free radical-mediated bromination. By adopting synthetic routes similar to those of intermediates **30**−**47**, intermediates **82**−**86** were generated, which were transformed to desired compounds **87**−**91** using a $NaBH(OAc)_3$-mediated reductive amination.

The syntheses of **93**, **94**, and **100** are shown in Scheme 3. The N-alkylation of intermediate **30** and $PhSO_2OMe$ generated intermediate **92**. Target molecules **93** and **94** were provided by $NaBH(OAc)_3$-mediated reductive amination of **92** and the appropriate amines. Compound **96** was synthesized by K_2CO_3-promoted O-alkylation of starting material **95** and 5-(chloromethyl)nicotinonitrile, which was transformed to benzyl chloride **97** using MsCl. N-alkylation of **97** and **98** was performed to afford intermediate **99**. Finally, the $NaBH(OAc)_3$-mediated reductive amination of **99** and D-serine provided desired compound **100**.

Biochemical Assay of PD-1/PD-L1 Blockade and SAR Study. At the beginning of our research, compounds **48**−**52**, which have different aryl groups, ether-linked groups, and tails, were synthesized to validate our design strategy. The inhibitory activities of the PD-1/PD-L1 interaction were evaluated at the biochemical level using a homogeneous time-resolved fluorescence (HTRF) assay. BMS202 was used as a positive control in the assay. As shown in Table 1, compounds **48**−**52** exhibited moderate inhibitory activities toward the PD-1/PD-L1 interaction, with IC_{50} values in the low- to mid-single-digit micromolar ranges. Comparisons of **52** with **49**, **50**, and **51** indicated that hydrophilic improvement of the tail resulted in higher inhibitory potency. In comparison with **49**, **48** showed comparable activity, indicating that aromatic heterocycles were also tolerated in this region. Notably, BMS202 showed an IC_{50} value of 0.175 μM in our HTRF assay, which was approximately 10-fold less than the value reported in the BMS patent.[25] Based on the above results, further optimizations were performed to improve inhibitor potency and investigate their SARs in a subsequent experiment.

The geometric differences of the linkers had a significant impact on the locations of the aryl group and ether-linked group, which affected their interaction with the PD-L1 homodimer and therefore the activity of the compounds. Based on this consideration, a series of compounds with diverse aryl groups and ether-linked groups were developed and evaluated. As summarized in Table 2, most of these compounds exhibited attractive inhibitory activity against the PD-1/PD-L1 interaction, with IC_{50} values in the nanomolar range. Compound **58** displayed the most potent activity, with an IC_{50} value of 12 nM. Comparisons of **52**, **53**, **54**, and **55** indicated that substitution at the aryl group was more beneficial to inhibitory activity than nonsubstitution; moreover, 2-Me substitution was more beneficial than 3-Me and 3-OMe substitution. Similar rules could be observed by

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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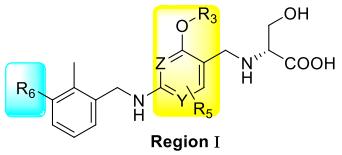
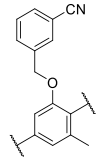
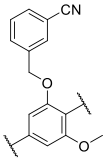
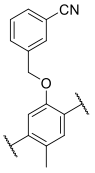
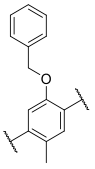
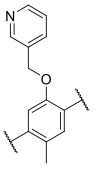
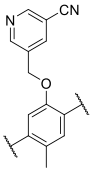
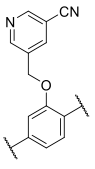
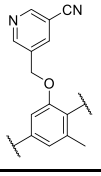
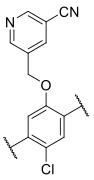
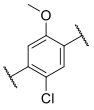
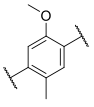
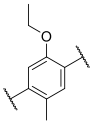
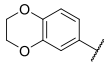
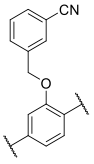
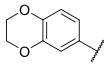
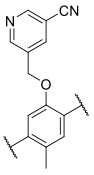
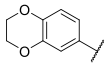
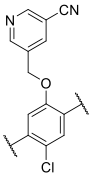
Table 2. Inhibitory Activities of Compounds 53−69 toward the PD-1/PD-L1 Interaction



53-69

Compound	R_6	Region I	IC_{50} (μM)[a]
53			0.363 ± 0.013
54			0.672 ± 0.067
55			0.165 ± 0.022
56			2.583 ± 0.351
57			0.092 ± 0.002
58			0.012±0.001
59			0.038 ± 0.003
60			0.436 ± 0.075
61			0.026 ± 0.001
62			4.536 ± 0.259
63			2.632 ± 0.076
64			0.107 ± 0.016
65			0.253 ± 0.003
66			0.347 ± 0.020
67			0.435 ± 0.017
68			0.044 ± 0.006
69			0.131 ± 0.005

[a]Data are displayed as averages of triplicate assays ± SD.

comparing **58**, **59**, and **60**. Compound **61** with 2-Cl substitution displayed a slightly lower inhibitory activity than that of **58**, with an IC_{50} value of 26 nM, suggesting that 2-Cl substitution was a useful modification. In general, the (3-cyanopyridin-5-yl)methyl moiety was the most preferred moiety for the ether-linked group region. Replacing the (3-

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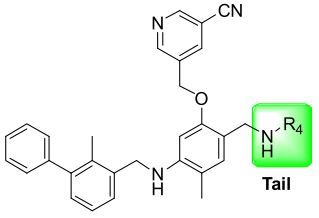
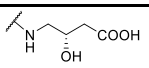
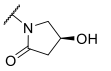
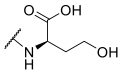
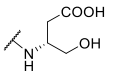
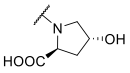
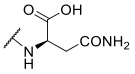
cyanopyridin-5-yl)methyl moiety with methyl, ethyl, benzyl, (pyridin-3-yl)methyl, and (3-cyanobenzene-5-yl)methyl moieties yielded **65**, **66**, **56**, **57**, and **55**, respectively, which showed reduced efficacy. Comparisons of **58** with **55** and **57** revealed that the N atom contributed slightly more than −CN to the high potency of the (3-cyanopyridin-5-yl)methyl moiety. Based on previous reports, the introduction of the 2,3-dihydro-1,4-benzodioxinyl moiety in the extension region resulted in the movement of Tyr56, which made the binding pocket of PD-L1 accessible to solvent on both sides and changed a deep hydrophobic cleft into a deep, hydrophobic tunnel.[27] Therefore, compounds **67**−**69** were synthesized to examine the impact of the introduced 2,3-dihydro-1,4-benzodioxinyl moiety on the efficacy of the compounds. Compounds **68** and **69** exhibited lower inhibitory activities than those of their precursor compounds (**58** and **61**), while compound **67** was more potent than compound **52**.

The hydrophilic tail provided a significant contribution to the observed high potencies by providing hydrogen bond interactions with PD-L1. A series of hydrophilic moieties bearing diverse structures were introduced to yield compounds **70**−**75**, as shown in Table 3. Compounds **70** and **72**−**75** exhibited promising efficacy as PD-1/PD-L1 blockades, with IC_{50} values below 50 nM, indicating that diverse scaffolds, such as linear, branched, and cyclic chains, and diverse hydrogen bond donors, such as hydroxyl, carboxyl, and amide moieties, were tolerated in the tail region. This discovery provided room to improve the potency and PK properties of the compounds. Compared to **70** and **74**, compound **71** was significantly less potent, indicating that two hydrogen bond donors were required for high efficacy.

Table 3. Inhibitory Activities of Compounds 70−75 toward the PD-1/PD-L1 Interaction



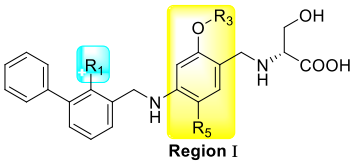
70-75

Compound	Tail	IC_{50} (μM)[a]
70		0.017 ± 0.001
71		1.334 ± 0.106
72		0.036 ± 0.004
73		0.023 ± 0.001
74		0.024 ± 0.002
75		0.047 ± 0.004

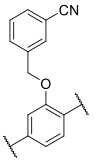
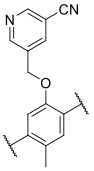
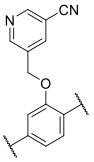
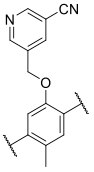
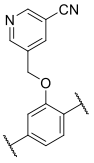
[a]Data are displayed as averages of triplicate assays ± SD.

To increase the hydrophobicity of the biaryl core, replacing methyl with −Br and $-CF_3$ provided **87**−**89** and **90**−**91**, respectively. As shown in Table 4, compounds **87**−**89** exhibited potencies comparable to those of their corresponding precursor compounds **52**, **58**, and **59**. However, **90** and **91** exhibited remarkably reduced efficacies compared with those of **58** and **59**, indicating that a strong electron-withdrawing group was unfavorable.

Table 4. Inhibitory Activities of Compounds 87−91 toward the PD-1/PD-L1 Interaction



87-91

Compound	R_1	Region I	IC_{50} (μM)[a]
87			0.637 ± 0.030
88			0.031 ± 0.004
89			0.206 ± 0.016
90			0.610 ± 0.038
91			2.933 ± 0.198

[a]Data are displayed as averages of triplicate assays ± SD.

Compounds **93**, **94**, and **100** were prepared to investigate whether other linear aliphatic amines were also desirable as linkers. As shown in Table 5, compound **94** was remarkably less potent than its precursor compound **50**; compound **93** was clearly inactive at concentrations up to 100 μM. The results indicated that using a N-substituted linear aliphatic amine as a linker was inappropriate. The N-substituted molecule probably formed an unfavorable conformation. In comparison to **59**,

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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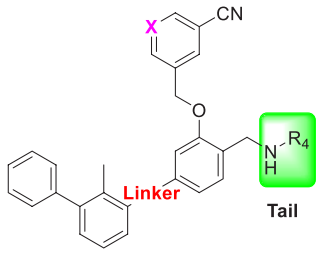
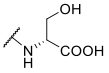
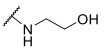
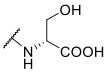
Table 5. Inhibitory Activities of Compounds 93, 94, and 100 toward the PD-1/PD-L1 Interaction



93, 94, 100

Compound	Linker	X	Tail	IC_{50} (μM)[a]
93		CH		>100
94		CH		22.55 ± 1.395
100		N		0.191 ± 0.027

[a]Data are displayed as averages of triplicate assays ± SD.

compound **100** displayed decreased activity, with an IC_{50} value of 191 nM, indicating that the direct linkage of an amine to the hydrophobic biaryl core was probably inappropriate.

Binding Specificity and Affinity of Compounds to Human PD-L1. To identify the binding target of these compounds that led to disruption of the interaction of PD-1 and PD-L1, a specificity assay was performed using the biolayer interferometry (BLI) technique. BLI is a sensitive and label-free technology for measuring biomolecular interactions. Human PD-L1/Fc and PD-1/Fc were loaded on an anti-human IgG Fc capture (AHC) sensor chip. The binding of the selected compound **52** with human PD-L1 (hPD-L1) and PD-1 (hPD-1) was measured at a concentration of 500 nM. As shown in Figure S1, **52** bound to PD-L1 and not to PD-1. To further verify whether the binding of compound **52** with hPD-L1 was specific, the binding of **52** with human PD-L2, CD47, TIM-3, CD27, and LAG-3 was examined. As shown in Figure S1, compound **52** showed no binding with these captured proteins on the sensor chip. These results indicated that this series of compounds specifically bound to hPD-L1.

Binding affinity is one of the most important indicators for evaluating the efficacy of binding. To evaluate the efficacy of small-molecule inhibitors that will have to compete with the natural protein partner of PD-L1, the binding affinities of **52**, **58**, **59**, **64**, BMS202, and hPD-1 to hPD-L1 were measured. The kinetic curves of association and dissociation are shown in Figure 4, in which the compounds show dose-dependent kinetic characteristics. The kinetic parameters and calculated binding affinities are summarized in Table 6. Analyses of the resulting binding kinetics revealed that the small-molecule inhibitors displayed high binding affinity with hPD-L1, with KD values in the nanomolar to picomolar ranges. Compound **58** exhibited the most potent affinity, with a KD value of 16.2 pM, which was 43 times greater than that of BMS202. hPD-1 exhibited >18-fold lower potency than those of the selected

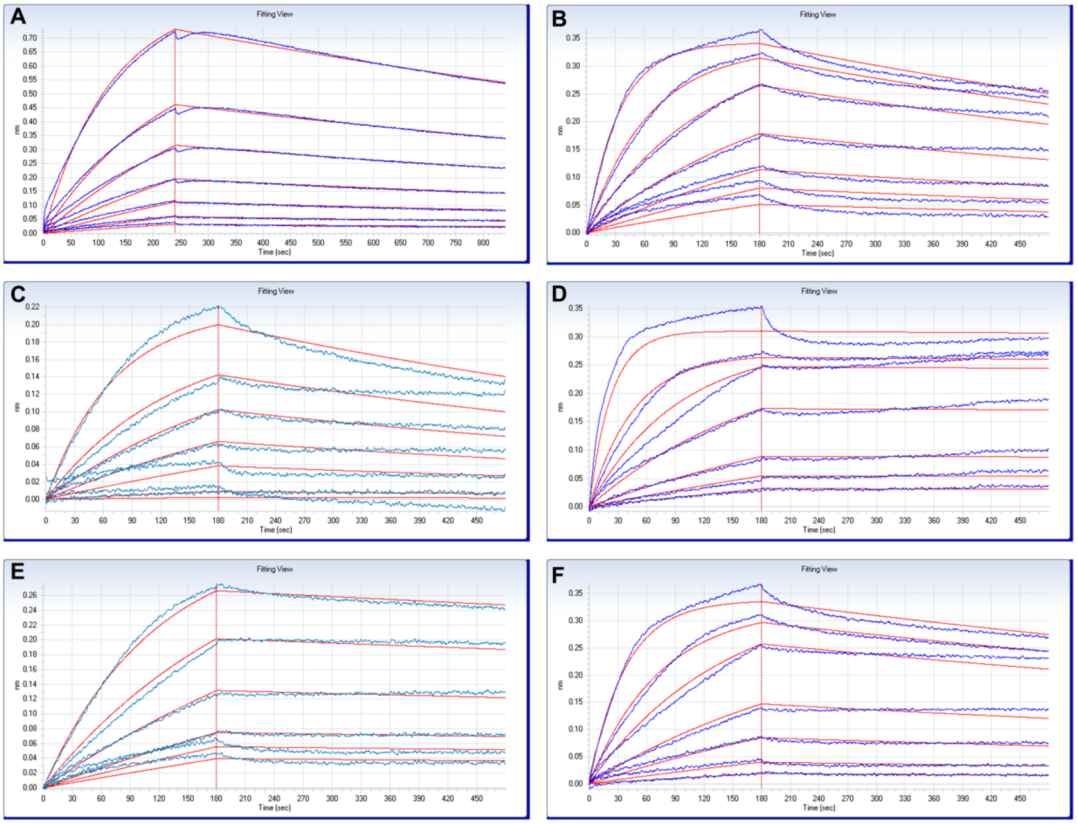


Figure 4. Association and dissociation kinetic curves of samples at seven different concentrations with hPD-L1. (A) hPD-1, (B) BMS202, (C) compd **52**, (D) compd **58**, (E) compd **59**, and (F) compd **64**.

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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Table 6. The Kinetic Parameters and Binding Affinities of Samples with hPD-L1[a]

samples	K_a (M^{-1} s^{-1})	K_{dis} (s^{-1})	KD (M)
hPD-1	1.54×10^4	5.03×10^{-4}	3.26×10^{-8}
BMS202	1.44×10^6	1.02×10^{-3}	7.06×10^{-10}
compd **52**	6.51×10^5	1.18×10^{-3}	1.81×10^{-9}
compd **58**	2.17×10^6	3.53×10^{-5}	1.62×10^{-11}
compd **59**	8.49×10^5	2.51×10^{-4}	2.96×10^{-10}
compd **64**	1.29×10^6	6.64×10^{-4}	5.15×10^{-10}

[a]Data are generated from seven different concentrations. k_a, association constant; k_{dis}, dissociation constant.

small-molecule inhibitors, with a KD value of 32.6 nM. The results demonstrated that these small-molecule inhibitors have high efficacy in competing with PD-1, the natural protein partner of PD-L1. These results were also consistent with the results from the HTRF assay.

Docking Analysis. Docking analysis was carried out to examine the binding mode of **58** with PD-L1 using GOLD software. The docking results showed that **58** docked well in the hydrophobic cleft formed by the PD-L1 dimer (Figure 5A) and superimposed well with BMS202 (Figure 5B). The serine tail of **58** was solvent-accessible and formed three hydrogen bonds with the Asp122 and Lys124 residues. The ether-linked nicotinonitrile group formed hydrogen bonds and $\pi-\pi$ interactions with Arg125 and Tyr123 residues. The distal phenyl of the biaryl core formed a T-shaped $\pi-\pi$ interaction with Tyr56. In addition, **58** established multiple key interactions with the Tyr56 residue of another monomer. The oxygen atom of the ether, linker, and aryl groups formed hydrogen bonds and $\pi-\sigma$ and $\pi-\pi$ interactions with Tyr56, respectively. Notably, the amine of the linker also formed a hydrogen bond with Ala121, which showed the contribution of the linker to inhibitor binding. The docking results indicated that **58** and the BMS compounds shared a similar PD-L1 binding mode, but the novel amine linker of **58** generated an additional interaction, indicating that the exploration of linkers was a useful strategy to develop novel inhibitors of PD-1/PD-L1.

Cell-Based PD-1/PD-L1 Blocking Assay. The binding and blocking ability of small-molecule inhibitors on the cellular level are crucial to their inhibitory efficiency. To investigate this, a cell-based PD-1/PD-L1 blocking assay was performed using hPD-L1-overexpressing CHO-K1 cells (CHO-K1-hPD-L1) and hPD-1 by flow cytometry. Briefly, CHO-K1-hPD-L1 cells were incubated with hPD-1, and then, binding was detected with an APC-labeled anti-human IgG antibody and measured by flow cytometry. The presence of small-molecule inhibitors would disrupt the binding of CHO-K1-hPD-L1 and hPD-1, leading to a change in the percentage of cells bound with hPD-1 (PCB). This change reflected the binding and blocking ability of the small-molecule inhibitors.

The binding and blocking ability of selected compounds BMS202, **52**, **58**, **59**, and **64** were measured in the cell-based PD-1/PD-L1 blocking assay at a concentration of 500 nM. The binding of CHO-K1-hPD-L1 cells and hPD-1 without an inhibitor was used as a positive control. The binding of normal CHO-K1 cells and hPD-1 was used as a negative control. As shown in Figure 6, the positive control group showed a higher PCB than that of the negative control group, indicating that the model was successful, and the increase in percentage was mediated by the PD-1/PD-L1 interaction. The groups with the synthesized compounds showed lower PCB values than that of the positive control group, clearly suggesting that the compounds had the ability to bind with hPD-L1 on the surface of CHO-K1 cells and competitively block the interaction of hPD-1 and hPD-L1. The calculated blocking efficacies of BMS202, **52**, **58**, **59**, and **64** were 99.2, 96.1, 97.5, 95.9, and 98.6%, respectively, which exhibited their potent blocking efficacy at the cellular level.

T Cell Function Assay. PD-1 and PD-L1 are the key coinhibitory signaling molecules that regulate the T cell function. Therefore, further functional verification of the compounds targeting the PD-1/PD-L1 interaction was required to evaluate their ability to regulate the T cell function, such as cytokine IFN-γ secretion. A coculture system of human peripheral blood mononuclear cells (PBMCs) and hPD-L1 was used to evaluate the effects of the compounds on T cell function regulation. PBMCs stimulated by anti-CD3 and anti-CD28 stimulatory antibodies could secrete IFN-γ. PD-L1 suppressed the T cell function by ligation with PD-1, leading to decreased production of IFN-γ. The compounds that inhibited the interaction of PD-1/PD-L1 could restore the T cell function, leading to enhanced production of IFN-γ. The immunological functions of selected compounds **52**, **58**, **59**, and **64** were evaluated using this coculture system. As shown in Figure 7, compounds **52**, **59**, and **64** showed no ability to promote IFN-γ secretion at a concentration of 0.1 μM. At 1 μM, **59** and **64** promoted the production of IFN-γ, which was increased by 42.5 and 40.9%, respectively, while **52** still

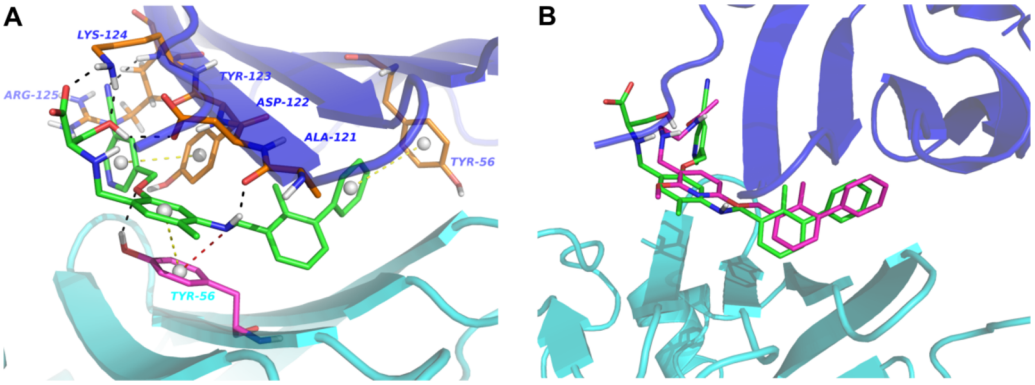

Figure 5. (A) Proposed binding mode of compound **58** with dimeric PD-L1 (the PDB code for dimeric PD-L1 protein is 5J89). (B) Binding overlap of compound **58** (green) and BMS202 (pink) in the binding site.

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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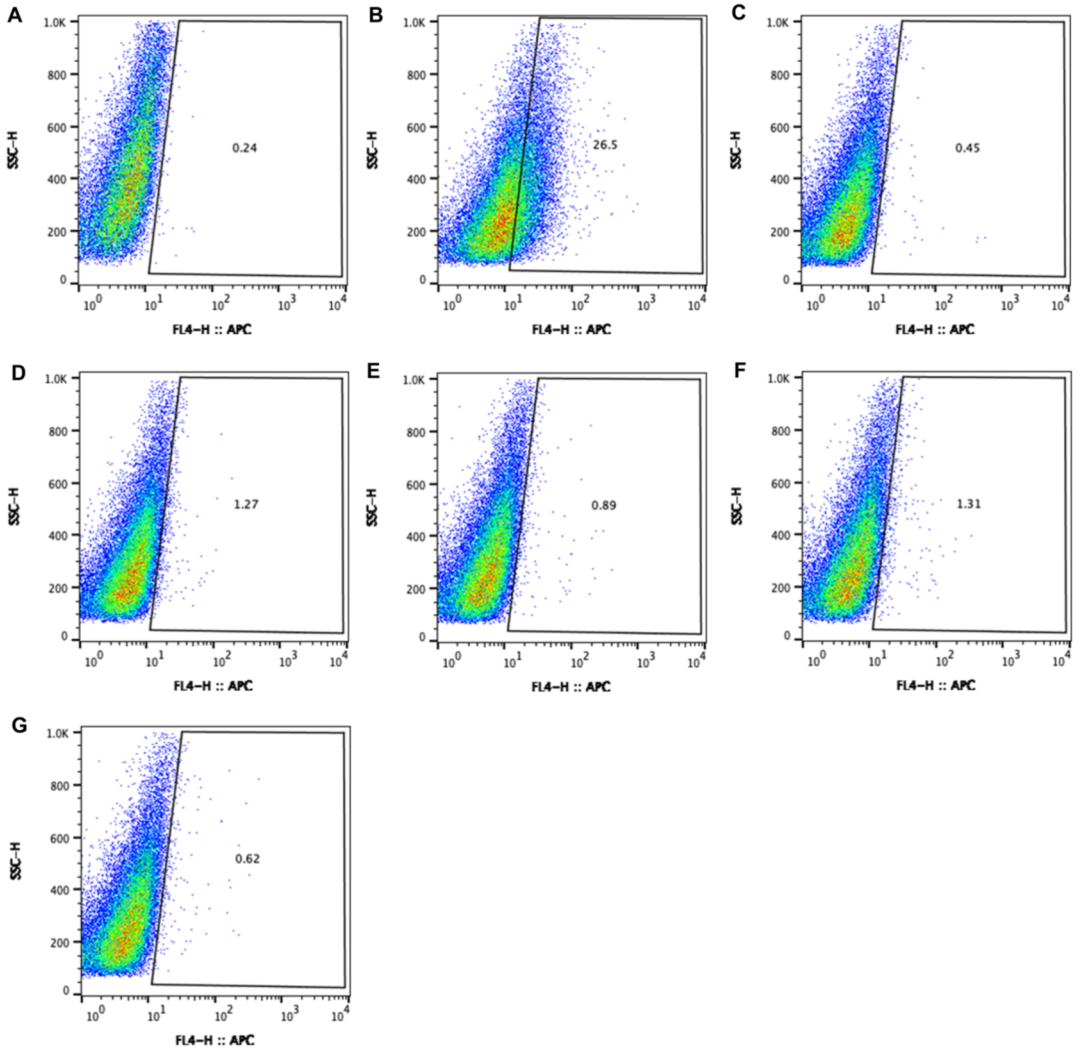

Figure 6. Representative flow cytometry plots: (A) negative control, (B) positive control, (C) BMS202, (D) compd **52**, (E) compd **58**, (F) compd **59**, and (G) compd **64**. The data in the graphs represent the percentage of cells bound with hPD-1.

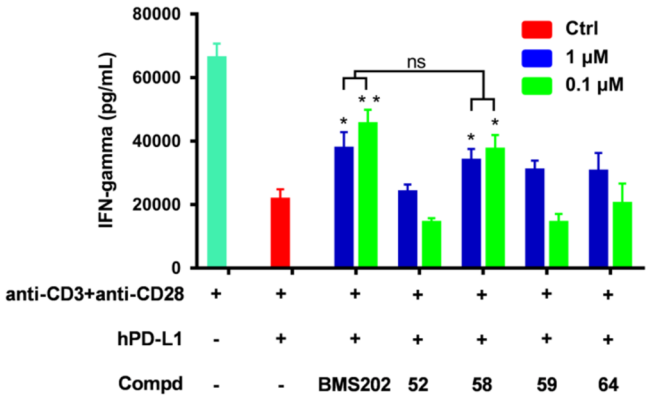

Figure 7. Effects of compounds BMS202, **52**, **58**, **59**, and **64** on IFN-γ secretion. Data are displayed as averages of quadruplicate assays ± SEM. *p < 0.05, **p < 0.01, and ns = no significance.

showed no efficacy because of the low inhibitory activity of PD-1/PD-L1. BMS202 significantly elevated the production of IFN-γ at concentrations of 0.1 and 1 μM, indicating that the assay system worked well. Compound **58** significantly promoted IFN-γ secretion at a concentration of 0.1 μM, which was increased by 72.9%. Treatment with 1 μM **58** increased the IFN-γ level by 56.8%. Because of the probable maximal dose effect, treatment with 1 μM **58** or BMS202 did not remarkably increase the IFN-γ level compared with treatment at 0.1 μM.

Evaluation of Cytotoxicity. Cytotoxicity significantly affects cellular functions, leading to inaccurate experimental results. To obtain accurate results, the cytotoxic effects of the tested compounds on human PBMCs were evaluated by the CellTiter-Glo luminescent cell viability assay. As shown in Figure 8, BMS202 and **48** showed high cytotoxicity to human PBMCs in a dose-dependent manner, with a killing rate of approximately 100% at a concentration of 100 μM, while **52**, **58**, **59**, and **64** showed no cytotoxicity up to 100 μM. At a concentration of 6.3 μM, all of the tested compounds showed no effect on cell growth, indicating that the compound concentrations used in the T cell function assay were appropriate. The compounds were further evaluated for cytotoxicity to Raji and RAW 264.7 cells. As shown in Figure 8, BMS202 and **48** also displayed high cytotoxicity to Raji and RAW 264.7 cells, with EC_{50} values of 13.51 and 13.84 μM and 10.08 and 6.97 μM, respectively. **52**, **58**, **59**, and **64** were not toxic to RAW 264.7 cells at a concentration below 100 μM. For Raji cells, **58** and **64** were not toxic at relatively low

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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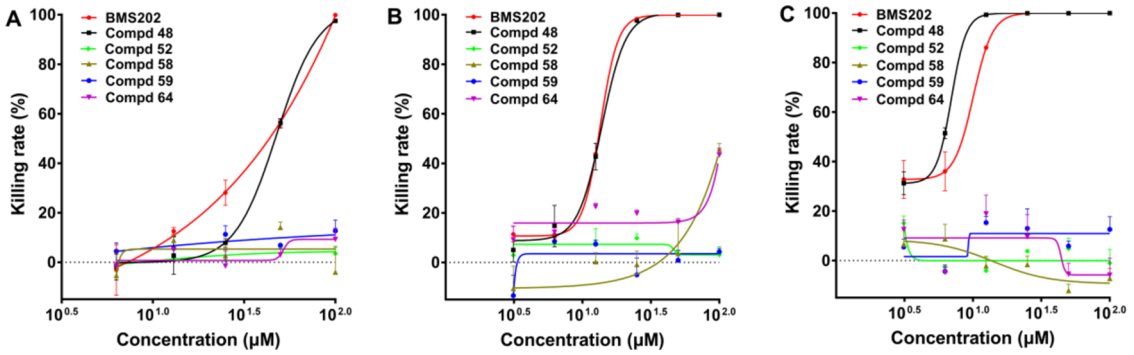

Figure 8. Cytotoxicity of compounds against human PBMCs (A), Raji cells (B), and RAW 264.7 cells (C). The experiment was performed in triplicate, and the values are expressed as the mean ± SEM.

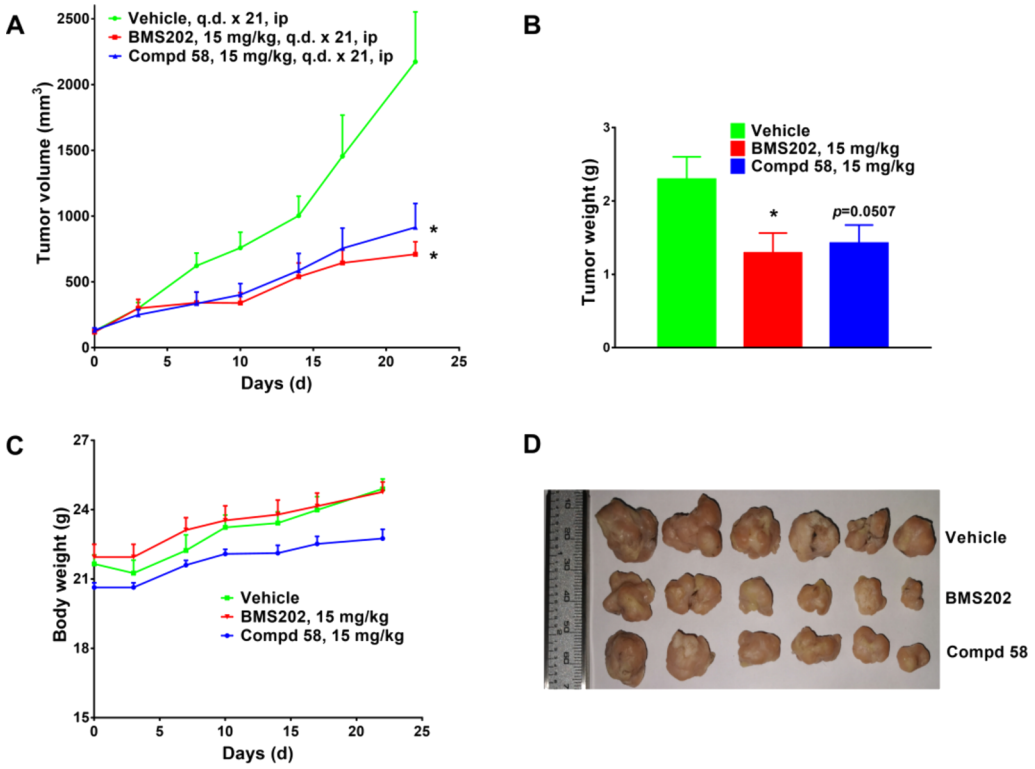

Figure 9. In vivo antitumor effect of compound **58** on a PD-1 humanized mouse model. (A) Tumor growth inhibition (the tumor volumes were measured and calculated once every 3−4 days). (B) Comparison of the final tumor weights in each group after 3 weeks of treatment. (C) Body weight of each group during the treatment period. The body weight was measured once every 3−4 days. (D) Resulting tumors excised from the animals after treatment. All data are presented as the mean ± SEM (6 female mice in each group). *$p < 0.05$.

concentrations and became toxic at relatively high concentrations, while **52** and **59** were not toxic.

In Vivo Antitumor Efficacy. Murine and human PD-1 share a sequence identity of 64%, and 77% of the PD-L1 sequence identity is shared.[38] To evaluate antitumor efficacy accurately, human PD-1 and PD-L1 are required in the murine model. On the other hand, based on the mechanism of action, the pharmacodynamic model of PD-1/PD-L1 inhibitors requires mice with a normal immune system. Based on the above considerations, PD-1-humanized mice and a PD-L1-genetically engineered MC38 mouse colon cancer cell line (mouse PD-L1 knockout, human PD-L1 knock-in) were used to establish a pharmacodynamic model.

On the basis of the excellent activities in vitro, compound **58** was selected to evaluate antitumor efficacy in vivo. BMS202 was used as a positive control. Compound **58** and BMS202 were administered by intraperitoneal injection once daily for 3 consecutive weeks at a dose of 15 mg/kg. As shown in Figure 9, the tumor volume of the vehicle group increased quickly and reached 2173.52 mm^3 after 21 days. Compound **58** and BMS202 significantly suppressed the tumor growth, with tumor growth inhibition (TGI) values of 61.7 and 71.1%, respectively. Compound **58** exhibited comparable antitumor efficacy in vivo when compared with BMS202, but BMS202 showed unspecific toxicity in the cytotoxic evaluation. In all experimental groups, no significant fluctuations in the body weight or unusual behaviors of mice were observed during the whole treatment period. All experimental treatments were well tolerated without mortality. These results confirmed the efficacy of compound **58** in vivo as a small-molecule inhibitor targeting the PD-1/PD-L1 interaction for cancer therapy.

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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Preliminary Evaluation for Pharmacokinetic Profiles. Because of the potent PD-1/PD-L1 inhibition in vitro and antitumor activity in vivo, an in vivo PK study was performed to preliminarily evaluate the druggability of **58** in male Sprague Dawley rats administered with 4 mg/kg iv (n = 6) and 15 mg/kg po (n = 6). The corresponding PK parameters are summarized in Table 7.

Table 7. Pharmacokinetic Parameters of Compound 58

	dosing route[c]	
PK parameters[a]	iv (4 mg/kg)	po (15 mg/kg)
$Cl_{\_obs}$ (mL/min/kg)	74.5 ± 10.1	NA[b]
$T_{1/2}$ (h)	1.38 ± 0.16	NA
C_0 (ng/mL)	4370 ± 1040	NA
AUC_{last} (h·ng/mL)	905 ± 117	NA
AUC_{Inf} (h·ng/mL)	908 ± 117	NA
$AUC_{\_\%Extrap\_obs}$ (%)	0.283 ± 0.124	NA
MRT_{Inf_obs} (h)	0.486 ± 0.069	NA
AUC_{last}/D (h·mg/mL)	226 ± 29	NA
V_{ss_obs} (L/kg)	2.17 ± 0.35	NA

[a]The values are expressed as the mean ± SD. [b]NA = not available. [c]Formulation: 25% MPD/10% RH40/65% water; 6 male rats in each group.

After iv administration of **58**, the values of C_0 and AUC_{Inf} achieved in plasma were 4370 ng/mL and 908 h·ng/mL, respectively, which indicated moderate systemic exposure. In addition, **58** showed a half-life ($T_{1/2}$) of 1.38 h, with a clearance (Cl_{obs}) of 74.5 mL/min/kg and a volume of distribution ($\bar{V}_{ss\_obs}$) of 2.17 L/kg. These results indicated that **58** showed moderate PK properties via intravenous injection, which provided a foundation for further development. Unfortunately, po administration of **58** exhibited very low exposures, with a plasma concentration below the quantifiable limit (0.5 ng/mL), indicating oral unavailability.

CONCLUSION

The PD-1/PD-L1 interaction has emerged as a promising target for cancer therapy. The approval of mAbs to inhibit the PD-1/PD-L1 interaction has proven the clinical significance of PD-1/PD-L1 inhibitors. Due to their advantages compared with mAbs, the discovery and development of small-molecule inhibitors of PD-1/PD-L1 have been of recent interest in medicinal chemistry. To develop novel small-molecule inhibitors of PD-1/PD-L1 for cancer therapy, a series of novel linear aliphatic amine-linked triaryl derivatives were designed, synthesized, and evaluated for their activities in vitro and in vivo. In the biochemical assay of PD-1/PD-L1 blockage, this novel chemical series showed potent inhibitory activities, with IC_{50} values in the nanomolar to micromolar ranges. SAR analysis indicated that hydrogen bond interactions with the tail were required for high potency; strong electron-withdrawing hydrophobic substituents in the biaryl core and N-substituents in the amine linker were unfavorable to inhibitory activity. A binding specificity assay indicated that this series of compounds specifically bound to PD-L1 and not to PD-1 or other checkpoints. Among the synthesized compounds, compound **58** was the most potent, with an IC_{50} value of 12 nM. Compound **58** also exhibited the most potent binding affinity with hPD-L1, with a KD value of 16.2 pM, which was approximately 2000-fold that of hPD-1, indicating its high efficacy for competing with the natural protein partner of PD-L1. The cell-based PD-1/PD-L1 blocking assay indicated that compound **58** could bind with hPD-L1 on the cellular surface and competitively block the interaction of hPD-1 with hPD-L1. In the T cell function assay, **58** restored the T cell function, leading to increased IFN-γ secretion. Evaluation of cytotoxicity showed that **58** was not toxic. Moreover, compound **58** significantly inhibited tumor growth in a humanized mouse model without obvious toxicity at a dose of 15 mg/kg. The results indicated the promising use of compound **58** as an alternative or complementary drug to mAbs for cancer treatment. Compound **58** showed moderate PK properties after intravenous injection in the PK study but did not exhibit oral bioavailability.

In conclusion, these studies provide biological evaluation models that may serve as useful tools for the development of small-molecule inhibitors of PD-1/PD-L1 and discovered compound **58**, which could be employed as a promising lead for further development of small-molecule inhibitors of PD-1/PD-L1 for cancer therapy. Currently, further optimization efforts to achieve new, potent small-molecule inhibitors of PD-1/PD-L1 with favorable oral PK profiles are underway in our laboratory.

EXPERIMENTAL SECTION

General Chemistry. Reagents and solvents were purchased from commercial suppliers and used without further purification. All the reactions were monitored by TLC using silica gel TLC plates (GF254). Silica gel (200−300 mesh) was used for chromatography. Melting points were determined on a Buchi melting point apparatus (M-565). The ^1H and ^{13}C NMR spectra were recorded on a JEOL-ECA400 spectrometer, with TMS as an internal standard at ambient temperature. All chemical shifts are reported in parts per million (ppm). All coupling constants were reported in Hertz. The ESI-MS was recorded on an Agilent TOF G6230A mass spectrometer. The purity of all final compounds was determined by HPLC on an Agilent 1260 system and was 95% or higher. HPLC Method A was performed on an Agilent ZORBAX SB-C8 column (4.6 mm × 250 mm, 5 μm) with ammonium acetate aqueous solution (10 mM) containing 5% MeCN as mobile phase A and MeCN containing 5% ammonium acetate aqueous solution (10 mM) as mobile phase B at a flow rate of 1 mL/min. The column temperature was maintained at 50 °C, and the UV wavelength for detection was 220 nm. The gradient program was as follows: 0−100% B (0−25 min), 100% B (25−30 min), and 0% B (30.01−35 min). HPLC Method B was performed on an Agilent ZORBAX Eclipse Plus-C18 column (4.6 mm × 100 mm, 3.5 μm) with 100% MeCN as mobile phase A and water (0.1% triethylamine and H_3PO_4 buffer solution, pH = 3) as mobile phase B at a flow rate of 1 mL/min. The column temperature was maintained at 35 °C, and the UV wavelength for detection was 220 nm. The gradient program was as follows: 60−5% B (0−10 min), 5% B (10−18 min), and 60% B (18.01−23 min). The final compounds were purified by preparative high-performance liquid chromatography on an Agilent 1260 Infinity system at ambient temperature. The method was performed on a Phenomenex Gemini-C18 column (30 mm × 100 mm, 5 μm) with 100% MeCN as mobile phase A and water as mobile phase B at a flow rate of 20 mL/min. The UV wavelength for detection was 254 nm. The gradient program was as follows: 40−100% A (0−25 min) and 40% A (25.01−30 min).

General Procedure A for Suzuki−Miyaura Coupling Reaction. At an argon atmosphere, halogenated benzene (1 equiv), phenylboronic acid (1.15−1.5 equiv) or (2,3-dihydrobenzo[b][1,4]-dioxin-6-yl)boronic acid (1.25 equiv), CsOAc (2.5 equiv), and $PdCl_2$dppf (0.1 equiv) were suspended in THF. The mixture was stirred at reflux for 23−36 h. Then, the mixture was cooled, concentrated in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water and

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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concentrated in vacuo. The crude product was purified by column chromatography.

*(2-Methyl-[1,1′-biphenyl]-3-yl)methanol (**11a**).* According to the general procedure A, **10** (2.51 g, 12.5 mmol) and phenylboronic acid (1.5 equiv) were used. The crude product was purified on a silica gel column (PE/EA = 10:1−7:1) to give **11a** (2.44 g, 98% yield) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44−7.32 (m, 4H), 7.32−7.27 (m, 2H), 7.26−7.23 (m, 1H), 7.20 (dd, J = 7.6, 1.2 Hz, 1H), 4.77 (s, 2H), 2.24 (s, 3H), 1.70 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.9, 142.1, 139.2, 133.6, 129.6, 129.4, 128.1, 126.9, 126.8, 125.6, 64.0, 15.9. ESI-MS: m/z = 181.11 $[M - OH]^+$.

*(3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylphenyl)-methanol (**11b**).* According to the general procedure A, **10** (3.25 g, 16.16 mmol) and (2,3-dihydrobenzo[b][1,4]dioxin-6-yl)boronic acid (1.25 equiv) were used. The crude product was purified on a silica gel column (PE/EA = 8:1−5:1) to give **11b** (4.05 g, 98% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (dd, J = 7.6, 1.2 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.17 (dd, J = 7.6, 1.6 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 8.0, 2.0 Hz, 1H), 4.76 (s, 2H), 4.30 (s, 4H), 2.25 (s, 3H), 1.66 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.0, 142.6, 142.3, 139.3, 135.5, 133.7, 129.6, 126.6, 125.6, 122.6, 118.3, 116.9, 64.5, 64.1, 16.0. ESI-MS: m/z = 257.12 $[M + H]^+$.

*3-(Bromomethyl)-2-methyl-1,1′-biphenyl (**12a**).* PBr_3 (0.36 mL, 3.81 mmol) was added dropwise to a solution of **11a** (1.51 g, 7.62 mmol) in DCM (30 mL) at 0 °C and stirred for further 20 min. The mixture was quenched by ice and extracted with DCM. The combined organic phase was washed with a saturated solution of $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness in vacuo to give **12a** (1.32 g, 66% yield) as a colorless oil, which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44−7.39 (m, 2H), 7.38−7.32 (m, 2H), 7.32−7.27 (m, 2H), 7.22 (d, J = 2.8 Hz, 1H), 7.21 (s, 1H), 4.60 (s, 2H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.4, 141.8, 136.4, 134.9, 130.8, 129.4, 129.3, 128.2, 127.0, 125.9, 33.3, 16.2. ESI-MS: m/z = 181.10 $[M - Br]^+$.

*6-(3-(Bromomethyl)-2-methylphenyl)-2,3-dihydrobenzo[b][1,4]-dioxine (**12b**).* According to a similar manner as described for the synthesis of **12a**, **11b** (513 mg, 2.0 mmol) was used to obtain **12b** (550 mg, 86% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35−7.29 (m, 1H), 7.22−7.15 (m, 2H), 6.93−6.89 (m, 1H), 6.82−6.80 (m, 1H), 6.78−6.74 (m, 1H), 4.59 (s, 2H), 4.31 (s, 4H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.1, 142.8, 142.7, 136.35, 135.2, 135.1, 130.8, 129.2, 125.9, 122.6, 118.3, 116.9, 64.5, 33.3, 16.2. ESI-MS: m/z = 239.11 $[M - Br]^+$.

*2-((2-Methyl-[1,1′-biphenyl]-3-yl)methyl)isoindoline-1,3-dione (**13a**).* At an argon atmosphere, DEAD (9.06 g, 52.00 mmol) was added dropwise to a solution of **11a** (7.36 g, 37.14 mmol), o-phthalimide (6.56 g, 44.57 mmol), and PPh_3 (13.64 g, 52.00 mmol) in anhydrous THF (170 mL) at 0 °C. After completion of addition, the mixture was stirred for 24 h at rt, and then concentrated to dryness in vacuo. The crude product was purified on a silica gel column (PE/DCM = 3:1−2:1) to give **13a** (11.21 g, 92% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92−7.86 (m, 2H), 7.77−7.72 (m, 2H), 7.43−7.38 (m, 2H), 7.36−7.31 (m, 1H), 7.30−7.26 (m, 2H), 7.24−7.21 (m, 1H), 7.20−7.12 (m, 2H), 4.94 (s, 2H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 143.0, 142.1, 134.6, 134.2, 133.4, 132.2, 129.5, 129.5, 128.1, 126.9, 126.7, 125.6, 123.5, 39.8, 16.6. ESI-MS: m/z = 328.13 $[M + H]^+$.

*2-(3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)-isoindoline-1,3-dione (**13b**).* According to a similar manner as described for the synthesis of **13a**, **11b** (3.46 g, 13.50 mmol) was used. The crude product was purified on a silica gel column (PE/DCM = 1:1−1:2) to give **13b** (5.02 g, 97% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89−7.87 (m, 2H), 7.75−7.73 (m, 2H), 7.21−7.16 (m, 1H), 7.14 (d, J = 4.0 Hz, 1H), 7.13 (s, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.74 (dd, J = 8.4, 2.0 Hz, 1H), 4.93 (s, 2H), 4.30 (s, 4H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 143.0, 142.6, 142.4, 135.5, 134.6, 134.1, 133.5, 132.2, 129.5, 126.5, 125.6, 123.5, 122.6, 118.3, 116.8, 64.5, 39.9, 16.6. ESI-MS: m/z = 386.14 $[M + H]^+$.

*(2-Methyl-[1,1′-biphenyl]-3-yl)methanamine (**14a**).* **13a** (11.21 g, 34.27 mmol) and $N_2H_4{\cdot}H_2O$ (85% w/w in water, 3.63 g, and 61.68 mmol) were suspended in EtOH (210 mL), stirred at reflux for 3 h, and then cooled to rt. To the reaction mixture, 6 N HCl (40 mL) was added and stirred for 30 min at reflux. The mixture was cooled to 0 °C and filtered. The filter cake was washed with EtOH a few times. The combined filtrate was alkalized (pH = 9−10), used 1 N NaOH at 0 °C, and extracted with DCM/MeOH (10:1). The combined organic phase was washed with H_2O once and concentrated to dryness in vacuo to give **14a** (6.5 g, 96% yield) as a colorless oil, which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43−7.37 (m, 2H), 7.36−7.31 (m, 2H), 7.30−7.27 (m, 2H), 7.24 (t, J = 7.6 Hz, 1H), 7.15 (dd, J = 7.6, 1.2 Hz, 1H), 3.93 (s, 2H), 2.22 (s, 3H), 1.79 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.9, 142.3, 141.3, 133.1, 129.4, 128.8, 128.1, 126.8, 126.4, 125.7, 44.6, 16.1. ESI-MS: m/z = 198.13 $[M + H]^+$.

*(3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylphenyl)-methanamine (**14b**).* According to a similar manner as described for the synthesis of **14a**, **13b** (4.88 g, 12.66 mmol) was used to obtain **14b** (3.20 g, 99% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (dd, J = 7.6, 1.2 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.12 (dd, J = 7.6, 1.2 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.75 (dd, J = 8.4, 2.0 Hz, 1H), 4.30 (s, 4H), 3.91 (s, 2H), 2.23 (s, 3H), 1.56 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.0, 142.5, 142.2, 141.5, 135.7, 133.2, 128.8, 126.2, 125.7, 122.6, 118.3, 116.8, 64.5, 44.7, 16.1. ESI-MS: m/z = 256.13 $[M + H]^+$.

*3-((5-Amino-2-formylphenoxy)methyl)benzonitrile (**15**).* 2-Hydroxy-4-nitrobenzaldehyde (4.81 g, 28.78 mmol), K_2CO_3 (5.97 g, 43.20 mmol), 3-(bromomethyl)benzonitrile (6.21 g, 31.68 mmol), and $(n\text{-}Bu)_4N^+{\cdot}I^-$ (50 mg, 0.14 mmol) were suspended in DMF (40 mL) and stirred for 4 h at rt. The reaction mixture was added to ice-water (900 mL). The precipitate was filtered, washed with water, and dried in vacuo. Then, the precipitate was suspended in cyclohexane, washed using an ultrasonic cleaner, and filtered to obtain 3-((2-formyl-5-nitrophenoxy)methyl)benzonitrile (**15-1**) (8.05 g, 99% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.49 (s, 1H), 8.09 (t, J = 1.6 Hz, 2H), 7.98−7.91 (m, 3H), 7.86 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.8 Hz, 1H), 5.51 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.8, 160.1, 151.7, 137.5, 132.3, 132.0, 130.9, 129.8, 129.6, 128.6, 118.7, 115.9, 111.6, 109.1, 69.5. ESI-MS: m/z = 281.06 $[M - H]^-$.

15-1 (8.05 g, 28.52 mmol), Fe (5.59 g, 99.82 mmol), and NH_4Cl (2.29 g, 42.81 mmol) were suspended in EtOH/H_2O (4/1, 100 mL) and stirred for 1.5 h at reflux. The reaction mixture was filtered. The filtrate was concentrated to dryness in vacuo. The residue was suspended in water and filtered to give **15** (6.48 g, 90% yield) as an orange solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.05 (s, 1H), 8.16−7.34 (m, 5H), 6.60−6.06 (m, 4H), 5.21 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.3, 162.5, 156.8, 138.5, 132.1, 131.7, 130.8, 130.2, 129.8, 118.7, 113.9, 111.5, 107.0, 95.8, 67.9. ESI-MS: m/z = 253.10 $[M + H]^+$.

*3-((5-Amino-2-formyl-3-methoxyphenoxy)methyl)benzonitrile (**16**).* 2-Methoxy-4-nitrobenzaldehyde (2.05 g, 11.32 mmol), 2-amino-4-chlorobenzoic acid (0.97 g, 5.66 mmol), 1-fluoro-2,4,6-trimethylpyrdinium triflate (4.91 g, 16.98 mmol), $Pd(OAc)_2$ (249 mg, 1.11 mmol), and TsOH (3.89 g, 22.59 mmol) were suspended in AcOH (80 mL) and stirred for 10 min at rt. Then, the mixture was stirred for further 24 h at 90 °C and concentrated to dryness in vacuo. The residue was purified by column chromatography to obtain 2-hydroxy-6-methoxy-4-nitrobenzaldehyde (**16-1**) (1.5 g, 67% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.06 (s, 1H), 10.41 (s, 1H), 7.39 (dd, J = 2.0, 0.8 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 4.03 (s, 3H). ESI-MS: m/z = 198.05 $[M + H]^+$.

According to a similar manner as described for the synthesis of compound **15-1**, **16-1** (684 mg, 3.47 mmol) was used to obtain 3-((2-formyl-3-methoxy-5-nitrophenoxy)methyl)benzonitrile (**16-2**) (1 g,

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93% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 1H), 8.00 (s, 1H), 7.91−7.80 (m, 2H), 7.66−7.57 (m, 3H), 5.42 (s, 2H), 3.99 (s, 3H).

According to a similar manner as described for the synthesis of compound **15**, **16-2** (900 mg, 2.88 mmol) was used, and the crude product was purified by column chromatography to obtain **16** (790 mg, 97% yield) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H), 8.03 (s, 1H), 7.88−7.83 (m, 1H), 7.80 (dt, J = 8.0, 1.4 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 6.39 (s, 2H), 5.87 (dd, J = 9.0, 1.4 Hz, 2H), 5.13 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.7, 164.2, 161.9, 156.6, 138.8, 131.6, 131.3, 130.3, 129.6, 118.8, 111.3, 104.2, 89.9, 88.9, 67.9, 55.4. ESI-MS: m/z = 283.11 [M + H]$^+$.

*5-((5-Amino-2-formylphenoxy)methyl)nicotinonitrile (**17**).* 2-Hydroxy-4-nitrobenzaldehyde (2.47 g, 14.78 mmol) and K_2CO_3 (2.15 g, 15.56 mmol) were suspended in DMF (30 mL) and stirred for 30 min at rt. Then, 5-(chloromethyl)nicotinonitrile (2.48 g, 16.29 mmol) and NaI (222 mg, 1.48 mmol) were added to the reaction mixture and stirred for 19 h at rt. The mixture was diluted with water (1 L), filtered, and washed with water. The filter cake was dried to give 5-((2-formyl-5-nitrophenoxy)methyl)nicotinonitrile (**17-1**) (4.05 g, 97% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.50 (s, 1H), 9.06 (d, J = 2.0 Hz, 1H), 9.05 (d, J = 2.0 Hz, 1H), 8.59 (t, J = 2.0 Hz, 1H), 8.12 (d, J = 1.2 Hz, 1H), 8.00−7.93 (m, 2H), 5.57 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.0, 159.9, 152.2, 152.1, 151.7, 138.8, 132.2, 129.5, 128.6, 116.8, 116.1, 109.1, 109.1, 67.5. ESI-MS: m/z = 284.07 [M + H]$^+$.

According to a similar manner as described for the synthesis of compound **15**, **17-1** (4.75 g, 16.77 mmol) was used to obtain **17** (4.12 g, 97% yield) as an orange solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 9.02 (s, 1H), 9.00 (s, 1H), 8.49 (s, 1H), 7.46 (d, J = 8.4 Hz, 1H), 6.43 (s, 2H), 6.26 (s, 2H), 5.24 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.5, 162.2, 156.7, 152.2, 151.8, 138.8, 133.0, 130.3, 116.8, 113.9, 109.0, 107.0, 95.8, 66.0. ESI-MS: m/z = 252.09 [M − H]$^-$.

*5-((5-Bromo-2-formyl-4-methylphenoxy)methyl)nicotinonitrile (**19**).* 4-Bromo-2-hydroxy-5-methylbenzaldehyde (430 mg, 2 mmol), K_2CO_3 (304 mg, 2.2 mmol), 5-(chloromethyl)nicotinonitrile (336 mg, 2.2 mmol), and NaI (30 mg, 0.2 mmol) were suspended in MeCN (6 mL) and stirred for 24 h at rt. The mixture was diluted with water (18 mL), filtered, and washed with water. The filter cake was dried to give **19** (649 mg, 98% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 9.03 (d, J = 1.6 Hz, 1H), 9.01 (d, J = 2.0 Hz, 1H), 8.53 (s, 1H), 7.68 (s, 1H), 7.63 (s, 1H), 5.40 (s, 2H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.4, 158.6, 152.7, 152.5, 139.3, 133.1, 132.5, 130.9, 130.0, 124.3, 118.3, 117.3, 116.0, 109.6, 67.6, 21.8. ESI-MS: m/z = 331.01 [M + H]$^+$.

*3-((5-Bromo-2-formyl-4-methylphenoxy)methyl)benzonitrile (**20**).* According to a similar manner as described for the synthesis of compound **19**, 4-bromo-2-hydroxy-5-methylbenzaldehyde (645 mg, 3 mmol) and 3-(bromomethyl)benzonitrile (647 mg, 3.3 mmol) were used. The crude product was suspended in cyclohexane, washed using an ultrasonic cleaner, and filtered to obtain **20** (801 mg, 81% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 8.01 (s, 1H), 7.85 (t, J = 8.6 Hz, 2H), 7.69−7.57 (m, 3H), 5.35 (s, 2H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.7, 158.2, 137.9, 132.2, 132.0, 131.8, 130.9, 130.2, 129.8, 129.5, 123.7, 118.7, 117.8, 111.5, 69.1, 21.3. ESI-MS: m/z = 330.00 [M + H]$^+$.

*4-Bromo-2-ethoxy-5-methylbenzaldehyde (**21**).* 4-Bromo-2-hydroxy-5-methylbenzaldehyde (660 mg, 3.07 mmol), K_2CO_3 (467 mg, 3.38 mmol), and CH_3CH_2I (527 mg, 3.38 mmol) were suspended in MeCN (15 mL) and stirred for 24 h at 60 °C. The mixture was diluted with water and extracted with EA. The combined organic phase was washed with water, concentrated in vacuo, and purified by column chromatography to obtain **21** (537 mg, 72% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.41 (s, 1H), 7.66 (s, 1H), 7.18 (s, 1H), 4.12 (q, J = 7.4 Hz, 2H), 2.35 (s, 3H), 1.47 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.1, 159.3, 132.7, 130.1, 129.3, 123.7, 116.7, 64.6, 21.7, 14.5. ESI-MS: m/z = 243.01 [M + H]$^+$.

*4-Bromo-5-methyl-2-(pyridin-3-ylmethoxy)benzaldehyde (**22**).* 4-Bromo-2-hydroxy-5-methylbenzaldehyde (430 mg, 2 mmol), K_2CO_3 (608 mg, 4.4 mmol), 3-(bromomethyl)pyridine hydrobromide (556 mg, 2.20 mmol), and NaI (30 mg, 0.2 mmol) were suspended in MeCN (10 mL) and stirred for 24 h at rt in a dark place. Then, another K_2CO_3 (332 mg, 2.4 mmol) and 3-(bromomethyl)pyridine hydrobromide (303 mg, 1.20 mmol) were added and stirred for further 12 h. The mixture was diluted with water and extracted with EA. The combined organic phase was washed with water, concentrated in vacuo, and purified by column chromatography to obtain **22** (180 mg, 29% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.41 (s, 1H), 8.71 (s, 1H), 8.64 (d, J = 4.0 Hz, 1H), 7.83−7.79 (m, 1H), 7.70 (s, 1H), 7.37 (dd, J = 8.0, 4.8 Hz, 1H), 7.29 (s, 1H), 5.17 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.6, 158.5, 150.0, 148.9, 135.3, 132.8, 131.3, 132.2, 129.9, 124.1, 123.8, 117.2, 68.5, 21.9. ESI-MS: m/z = 306.01 [M + H]$^+$.

*2-(Benzyloxy)-4-bromo-5-methylbenzaldehyde (**23**).* According to a similar manner as described for the synthesis of compound **19**, 4-bromo-2-hydroxy-5-methylbenzaldehyde (430 mg, 2 mmol) and benzyl bromide (376 mg, 2.2 mmol) were used to obtain **23** (503 mg, 82% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.45 (s, 1H), 7.69 (s, 1H), 7.45−7.33 (m, 5H), 7.28 (s, 1H), 5.14 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.0, 159.1, 135.6, 132.8, 130.9, 129.6, 128.9, 128.5, 127.5, 124.2, 117.4, 71.0, 21.9. ESI-MS: m/z = 327.00 [M + Na]$^+$.

*5-((5-Bromo-4-chloro-2-formylphenoxy)methyl)nicotinonitrile (**24**).* According to a similar manner as described for the synthesis of compound **16**, 4-bromo-3-chlorobenzaldehyde (2.53 g, 11.52 mmol) was used to obtain 4-bromo-5-chloro-2-hydroxybenzaldehyde (**24-1**) (1.50 g, 55% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 10.20 (s, 1H), 7.73 (s, 1H), 7.38 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.7, 159.3, 129.0, 128.7, 123.7, 123.2, 122.4. ESI-MS: m/z = 234.90 [M + H]$^+$.

According to a similar manner as described for the synthesis of compound **19**, **24-1** (471 mg, 2 mmol) was used to obtain **24** (663 mg, 94% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H), 9.04 (d, J = 2.0 Hz, 1H), 9.01 (d, J = 2.0 Hz, 1H), 8.54 (t, J = 2.0 Hz, 1H), 7.85 (s, 1H), 7.82 (s, 1H), 5.45 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.0, 158.2, 152.2, 152.0, 138.8, 132.2, 129.3, 128.7, 126.1, 125.0, 119.7, 116.8, 109.1, 67.5. ESI-MS: m/z = 350.95 [M + H]$^+$.

*3-((3-Cyanobenzyl)oxy)-4-formyl-5-methylphenyl Trifluoromethanesulfonate (**25**).* The solution of Tf_2O (2.96 g, 10.5 mmol) in DCM (15 mL) was added dropwise to a solution of 2,4-dihydroxy-6-methylbenzaldehyde (1.52 g, 10 mmol), 2,6-lutidine (1.13 g, 10.5 mmol), and DMAP (245 mg, 2 mmol) in DCM (40 mL) at 0 °C. After completion of addition, the mixture was stirred for 10 h at rt, diluted with water, and extracted with DCM. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified by column chromatography to obtain 4-formyl-3-hydroxy-5-methylphenyl trifluoromethanesulfonate (**25-1**) (1.7 g, 60% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.16 (s, 1H), 10.31 (s, 1H), 6.76 (d, J = 2.4 Hz, 1H), 6.67 (d, J = 2.4 Hz, 1H), 2.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.5, 164.8, 154.4, 145.3, 118.7 (q, J = 319.2 Hz), 118.1, 114.9, 109.1, 18.5. ESI-MS: m/z = 285.00 [M + H]$^+$.

25-1 (220 mg, 0.77 mmol), K_2CO_3 (128 mg, 0.92 mmol), 3-(bromomethyl)benzonitrile (159 mg, 0.81 mmol), and $(n\text{-Bu})_4N^+\cdot I^-$ (15 mg, 0.04 mmol) were suspended in MeCN (8 mL) and stirred for 6 h at rt. The mixture was diluted with water and extracted with DCM. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified by column chromatography to obtain **25** (286 mg, 93% yield) as a yellowish

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.64 (s, 1H), 7.78−7.61 (m, 3H), 7.60−7.52 (m, 1H), 6.80 (s, 2H), 5.20 (s, 2H), 2.63 (s, 3H), (isomer ratio = 5:1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.2, 162.4, 152.3, 145.3, 136.6, 132.4, 131.7, 130.8, 129.9, 123.3, 118.7 (q, J = 319.6 Hz), 118.3, 117.2, 113.2, 104.0, 70.0, 21.8. ESI-MS: m/z = 400.03 $[M + H]^+$.

*3-((5-Cyanopyridin-3-yl)methoxy)-4-formyl-5-methylphenyl Trifluoromethanesulfonate (**26**).* **25-1** (852 mg, 3 mmol), K_2CO_3 (954 mg, 6.90 mmol), 5-(chloromethyl)nicotinonitrile hydrochloride (624 mg, 3.30 mmol), and NaI (45 mg, 0.3 mmol) were suspended in MeCN (24 mL), stirred for 12 h at rt, and stirred for further 9 h at 45 °C. The mixture was diluted with water and extracted with EA. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified by column chromatography to obtain **26** (780 mg, 65% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.61 (s, 1H), 8.93 (s, 2H), 8.10 (s, 1H), 6.85 (s, 2H), 5.25 (s, 2H), 2.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.7, 161.9, 152.5, 152.3, 151.9, 145.6, 138.3, 131.6, 123.3, 118.7 (q, J = 319 Hz), 117.7, 116.1, 110.5, 104.0, 67.7, 21.7. ESI-MS: m/z = 401.03 $[M + H]^+$.

*3-((2-Formyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-phenoxy)methyl)benzonitrile (**30**).* **11a** (490 mg, 1.88 mmol), **15** (567 mg, 2.25 mmol), and K_2CO_3 (392 mg, 2.84 mmol) were suspended in MeCN/DMF (15 mL/2 mL) and stirred for 11 h at reflux. Then, the mixture was cooled, concentrated in vacuo, diluted with water, and extracted with EA. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified on a silica gel column (DCM/EA = 50:1) to give **30** (460 mg, 57% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.23 (s, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.72−7.66 (m, 2H), 7.66−7.60 (m, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.48−7.40 (m, 2H), 7.40−7.35 (m, 1H), 7.33−7.27 (m, 3H), 7.25−7.21 (m, 2H), 6.32 (dd, J = 8.8, 2.0 Hz, 1H), 6.09 (d, J = 2.0 Hz, 1H), 5.14 (s, 2H), 4.67 (br, 1H), 4.40 (d, J = 4.8 Hz, 2H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.0, 162.7, 154.8, 143.3, 141.8, 138.0, 135.9, 133.8, 131.8, 131.4, 131.2, 130.5, 129.8, 129.7, 129.4, 128.2, 127.5, 127.1, 125.9, 118.6, 116.1, 112.8, 106.1, 94.9, 68.9, 46.5, 16.3. ESI-MS: m/z = 433.19 $[M + H]^+$.

*3-((2-Formyl-3-methoxy-5-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)phenoxy)methyl)benzonitrile (**31**).* **11a** (290 mg, 1.11 mmol), **16** (330 mg, 1.17 mmol), K_2CO_3 (184 mg, 1.33 mmol), and DMF (5 mL) were added to a sealed vial. The mixture was heated to 85 °C and held for 35 min in a microwave reactor. After being cooled to room temperature, the reaction mixture was diluted with H_2O and extracted with DCM. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified on a silica gel column (PE/EA = 1:1) to give **31** (150 mg, 29% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.32 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.70 (s, 1H), 7.59 (dt, J = 7.6, 1.4 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.45−7.40 (m, 2H), 7.40−7.34 (m, 1H), 7.32−7.27 (m, 3H), 7.24 (d, J = 2.0 Hz, 1H), 7.23 (s, 1H), 5.78 (dd, J = 18.2, 1.8 Hz, 2H), 5.11 (s, 2H), 4.69 (br, 1H), 4.39 (s, 2H), 3.85 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.4, 164.6, 162.8, 154.8, 143.3, 141.8, 138.2, 135.9, 133.8, 131.5, 131.2, 130.1, 129.9, 129.5, 129.3, 128.2, 127.6, 127.1, 125.9, 118.7, 112.6, 106.3, 89.1, 88.3, 69.1, 55.8, 46.4, 16.3. ESI-MS: m/z = 463.19 $[M + H]^+$.

*5-((2-Formyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-phenoxy)methyl)nicotinonitrile (**32**).* According to a similar manner as described for the synthesis of **30**, **11a** (2.48 g, 9.50 mmol), **17** (2.52 g, 9.95 mmol), and K_2CO_3 (1.58 g, 11.4 mmol) were suspended in MeCN/DMF (51 mL/17 mL) and stirred for 10 h at 60 °C. The crude product was purified on a silica gel column (DCM/EA = 10:1) to give **32** (1 g, 24% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.06 (s, 1H), 9.00 (d, J = 2.0 Hz, 1H), 8.98 (d, J = 2.0 Hz, 1H), 8.48 (t, J = 2.0 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.49−7.43 (m, 2H), 7.40−7.35 (m, 2H), 7.33−7.30 (m, 2H), 7.28 (dd, J = 7.6, 1.2 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.14 (dd, J = 7.4, 1.4 Hz, 1H), 6.38 (s, 1H), 6.37 (s, 1H), 5.29 (s, 2H), 4.41 (d, J = 5.2 Hz, 2H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.7, 162.2, 155.8, 152.2, 151.8, 142.1, 141.5, 138.8, 136.9, 133.4, 133.0, 130.1, 129.2, 128.8, 128.2, 127.0, 126.9, 125.5, 116.9, 114.2, 109.0, 105.7, 94.6, 66.1, 45.0, 15.9. ESI-MS: m/z = 434.19 $[M + H]^+$.

*5-Chloro-2-methoxy-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)benzaldehyde (**33**).* According to a similar manner as described for the synthesis of **30**, **11a** (418 mg, 1.6 mmol), 4-amino-5-chloro-2-methoxybenzaldehyde (compd **18**, 312 mg, 1.68 mmol), and K_2CO_3 (265 mg, 1.92 mmol) were suspended in MeCN (15 mL) and stirred for 18 h at reflux. The crude product was purified on a silica gel column (PE/DCM = 1:1.5) to give **33** (277 mg, 47% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.14 (s, 1H), 7.79 (s, 1H), 7.47−7.40 (m, 2H), 7.40−7.34 (m, 1H), 7.34−7.28 (m, 3H), 7.28−7.23 (m, 2H), 6.14 (s, 1H), 5.22 (br, 1H), 4.48 (s, 2H), 3.87 (s, 3H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.7, 162.9, 149.7, 143.4, 141.7, 135.3, 133.9, 130.0, 129.4, 129.0, 128.2, 127.6, 127.1, 126.0, 115.5, 111.9, 93.0, 55.8, 46.6, 16.4. ESI-MS: m/z = 366.13 $[M + H]^+$.

*3-((5-((3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)-amino)-2-formylphenoxy)methyl)benzonitrile (**34**).* According to a similar manner as described for the synthesis of **30**, **11b** (550 mg, 1.72 mmol), **15** (435 mg, 1.72 mmol), and K_2CO_3 (262 mg, 1.90 mmol) were suspended in MeCN/DMF (10 mL/3 mL) and stirred for 11 h at reflux. The crude product was purified on a silica gel column (PE/EA = 2:1) to give **34** (170 mg, 20% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.23 (s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.71−7.66 (m, 2H), 7.63 (d, J = 7.8 Hz, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.25−7.22 (m, 1H), 7.22−7.17 (m, 2H), 6.91 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 8.2, 2.2 Hz, 1H), 6.31 (dd, J = 8.6, 1.8 Hz, 1H), 6.07 (d, J = 1.6 Hz, 1H), 5.14 (s, 2H), 4.64 (s, 1H), 4.38 (s, 2H), 4.31 (s, 4H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.2, 162.8, 154.9, 143.2, 142.8, 142.8, 138.1, 135.9, 135.2, 134.0, 132.0, 131.5, 131.3, 130.6, 130.0, 129.8, 127.4, 126.0, 122.6, 118.7, 118.3, 117.1, 116.17, 112.9, 106.2, 95.0, 69.0, 64.6, 46.6, 16.4. ESI-MS: m/z = 491.20 $[M + H]^+$.

General Procedure B for Buchwald−Hartwig Coupling Reaction. At an argon atmosphere, electrophile/amines = 1:1.2 equiv or 1.2:1 equiv, Cs_2CO_3 (1.5 equiv), BINAP (0.2 equiv), and $Pd(OAc)_2$ (0.1 equiv) were suspended in dioxane. The mixture was stirred at 85 °C or reflux for 16−24 h. Then, the mixture was cooled, concentrated in vacuo, diluted with water, and extracted with DCM or EA. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified by column chromatography.

*5-((2-Formyl-4-methyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)phenoxy)methyl)nicotinonitrile (**35**).* According to the general procedure B, **14a** (296 mg, 1.5 mmol) and **19** (596 mg, 1.8 mmol) were used and stirred for 20 h at reflux. The crude product was purified on a silica gel column (PE/EA = 1.5:1) to give **35** (300 mg, 45% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.21 (s, 1H), 8.84 (t, J = 1.8 Hz, 2H), 8.05 (t, J = 2.0 Hz, 1H), 7.61 (s, 1H), 7.46−7.42 (m, 2H), 7.39−7.35 (m, 1H), 7.33−7.30 (m, 2H), 7.27−7.24 (m, 3H), 6.08 (s, 1H), 5.18 (s, 2H), 4.50 (br, 1H), 4.44 (s, 2H), 2.25 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.6, 161.1, 153.5, 151.9, 151.7, 142.0, 141.6, 138.4, 136.9, 133.1, 132.8, 129.2, 129.1, 128.4, 128.1, 126.8, 125.8, 125.3, 116.8, 115.2, 113.6, 108.9, 93.3, 66.3, 44.8, 16.9, 15.9. ESI-MS: m/z = 448.21 $[M + H]^+$.

*3-((2-Formyl-3-methyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)phenoxy)methyl)benzonitrile (**36**).* According to the general procedure B, **14a** (99 mg, 0.5 mmol) and **25** (200 mg, 0.5 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (DCM/EA = 200:1) to give **36** (131 mg, 59% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.46 (s, 1H), 7.69−7.64 (m, 2H), 7.63−7.59 (m, 1H), 7.53−7.46 (m, 1H), 7.46−7.40 (m, 2H), 7.39−7.34 (m, 1H), 7.32−7.27 (m, 3H), 7.24 (d, J = 2.0 Hz, 1H), 7.23 (s, 1H), 6.10 (s, 1H), 5.99 (d, J = 2.0 Hz, 1H), 5.11 (s, 2H), 4.53 (br, 1H), 4.38 (d, J = 3.2 Hz, 2H), 2.57 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.9, 164.2, 153.3, 145.0, 143.2, 141.8, 138.1, 136.0, 133.8, 131.8, 131.4, 130.4, 129.8, 129.6, 129.3, 128.2, 127.5, 127.0, 125.9, 118.6,

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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114.4, 112.8, 108.6, 93.3, 69.1, 46.3, 22.5, 16.3. ESI-MS: m/z = 447.17 $[M + H]^+$.

*5-((2-Formyl-3-methyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)phenoxy)methyl)nicotinonitrile (**37**).* According to the general procedure B, **14a** (311 mg, 1.58 mmol) and **26** (760 mg, 1.89 mmol) were used and stirred for 20 h at 85 °C. The crude product was purified on a silica gel column (DCM/MeOH = 100:1) to give **37** (305 mg, 43% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.27 (s, 1H), 9.00 (d, J = 2.0 Hz, 1H), 8.96 (d, J = 2.0 Hz, 1H), 8.45 (t, J = 2.0 Hz, 1H), 7.49−7.42 (m, 2H), 7.42−7.34 (m, 1H), 7.34−7.29 (m, 2H), 7.29−7.18 (m, 3H), 7.14 (dd, J = 7.4, 1.4 Hz, 1H), 6.27 (s, 1H), 6.16 (s, 1H), 5.26 (s, 2H), 4.39 (d, J = 5.2 Hz, 2H), 2.41 (s, 3H), 2.19 (s, 3H). ESI-MS: m/z = 448.20 $[M + H]^+$.

*2-Methoxy-6-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-nicotinaldehyde (**38**).* BOC_2O (553 mg, 2.54 mmol) was added to a solution of **14a** (1 g, 5.07 mmol) and DABCO (57 mg, 0.51 mmol) in DCM (30 mL) at rt and stirred overnight. The mixture was diluted with n-hexane (30 mL). The precipitate was filtered and washed with n-hexane/DCM (1:1) to yield 1,3-bis((2-methyl-[1,1′-biphenyl]-3-yl)methyl)urea (660 mg, 62% yield) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46−7.40 (m, 2H), 7.40−7.34 (m, 1H), 7.31−7.26 (m, 3H), 7.23 (t, J = 7.4 Hz, 1H), 7.09 (dd, J = 7.2, 1.6 Hz, 1H), 6.39 (t, J = 5.8 Hz, 1H), 4.29 (d, J = 6.0 Hz, 2H), 2.14 (s, 3H). ESI-MS: m/z = 421.21 $[M + H]^+$.

At an argon atmosphere, 1,3-bis((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)urea (907 mg, 2.16 mmol), 6-chloro-2-methoxynicotinaldehyde (compd **27**, 814 mg, 4.75 mmol), Cs_2CO_3 (2.10 g, 6.48 mmol), s-phos (266 mg, 0.65 mmol), and $Pd(OAc)_2$ (73 mg, 0.33 mmol) were suspended in dioxane (25 mL) and stirred for 24 h at reflux. Then, the mixture was cooled, concentrated in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water, and the precipitate was filtered out. The filtrate was concentrated in vacuo and purified by silica gel column chromatography (PE/EA = 8:1) and preparative thin-layer chromatography (PE/ DCM/EA = 10:5:1) to give **38** (364 mg, 25% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.07 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.45−7.39 (m, 2H), 7.38−7.33 (m, 1H), 7.33−7.27 (m, 3H), 7.25−7.19 (m, 2H), 6.05 (d, J = 8.4 Hz, 1H), 5.32 (br, 1H), 4.65 (s, 2H), 3.98 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.1, 166.0, 160.8, 143.2, 141.9, 139.1, 136.4, 133.9, 129.7, 129.4, 128.2, 127.7, 127.0, 125.8, 116.0, 103.9, 53.4, 44.7, 16.4. ESI-MS: m/z = 333.14 $[M + H]^+$.

*4-Methoxy-6-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-nicotinaldehyde (**39**).* According to a similar manner as described for the synthesis of **38**, 1,3-bis((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-urea (660 mg, 1.57 mmol) and 6-chloro-4-methoxynicotinaldehyde (compd **28**, 448 mg, 2.61 mmol) were used. The crude product was purified by silica gel column chromatography (PE/EA = 2.5:1) and preparative thin-layer chromatography (DCM/MeOH = 40:1) to give **39** (143 mg, 16% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.04 (s, 1H), 8.38 (s, 1H), 7.44−7.39 (m, 2H), 7.38−7.33 (m, 1H), 7.31 (dd, J = 6.8, 2.4 Hz, 1H), 7.29−7.26 (m, 2H), 7.25−7.20 (m, 2H), 5.81 (s, 1H), 5.77 (br, 1H), 4.59 (d, J = 5.2 Hz, 2H), 3.89 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.3, 168.0, 163.3, 153.6, 143.3, 141.8, 135.9, 134.0, 129.9, 129.3, 128.2, 127.6, 127.0, 125.9, 114.1, 86.5, 55.5, 45.3, 16.4. ESI-MS: m/z = 333.16 $[M + H]^+$.

*5-((4-Chloro-2-formyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)phenoxy)methyl)nicotinonitrile (**40**).* According to the general procedure B, **14a** (354 mg, 1.80 mmol) and **24** (527 mg, 1.50 mmol) were used and stirred for 24 h at 85 °C. The crude product was purified on a silica gel column (PE/EA = 3:1−2:1) to give **40** (345 mg, 49% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.05 (s, 1H), 8.93 (d, J = 2.0 Hz, 1H), 8.86 (d, J = 2.0 Hz, 1H), 8.37 (t, J = 2.0 Hz, 1H), 7.61 (s, 1H), 7.49−7.43 (m, 2H), 7.41−7.35 (m, 1H), 7.35−7.31 (m, 2H), 7.19−7.14 (m, 1H), 7.11 (s, 1H), 7.11−7.08 (m, 2H), 6.29 (s, 1H), 5.27 (s, 2H), 4.58 (d, J = 6.0 Hz, 2H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.4, 160.8, 151.9, 151.8, 150.2, 142.1, 141.5, 138.5, 136.4, 132.7, 132.6, 129.2, 128.5, 128.4, 128.2, 126.9, 125.4, 125.4, 116.8, 114.5, 111.3, 109.0, 95.2, 66.7, 44.6, 15.9. ESI-MS: m/z = 468.13 $[M + H]^+$.

*2-Methoxy-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)benzaldehyde (**41**).* According to the general procedure B, **14a** (473 mg, 2.40 mmol) and 4-bromo-2-methoxy-5-methylbenzaldehyde (compound **29**, 458 mg, 2.0 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (PE/EA = 7:1−5:1) to give **41** (405 mg, 59% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 1H), 7.47−7.41 (m, 2H), 7.40−7.32 (m, 2H), 7.33−7.25 (m, 3H), 7.22 (t, J = 7.6 Hz, 1H), 7.11 (dd, J = 7.2, 1.2 Hz, 1H), 6.69 (t, J = 5.8 Hz, 1H), 6.05 (s, 1H), 4.54 (d, J = 5.6 Hz, 2H), 3.71 (s, 3H), 2.25 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.2, 162.7, 153.6, 142.1, 141.6, 137.2, 132.9, 129.2, 128.7, 128.4, 128.2, 126.8, 126.3, 125.4, 114.4, 113.1, 91.9, 55.3, 44.8, 16.8, 16.0. ESI-MS: m/z = 346.18 $[M + H]^+$.

*2-(Benzyloxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)benzaldehyde (**42**).* According to the general procedure B, **14a** (367 mg, 1.86 mmol) and **23** (473 mg, 1.55 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (PE/EA = 7:1) to give **42** (337 mg, 52% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.29 (s, 1H), 7.60 (d, J = 0.8 Hz, 1H), 7.45−7.41 (m, 2H), 7.40−7.33 (m, 5H), 7.33−7.29 (m, 3H), 7.29−7.26 (m, 1H), 7.25−7.23 (m, 2H), 6.14 (s, 1H), 5.12 (s, 2H), 4.39 (s, 2H), 4.34 (br, 1H), 2.22 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.6, 162.5, 152.8, 143.3, 141.8, 136.6, 135.9, 133.8, 129.8, 129.7, 129.4, 128.7, 128.2, 128.1, 127.5, 127.2, 127.0, 125.9, 115.3, 114.5, 93.6, 70.5, 46.7, 16.5, 16.3. ESI-MS: m/z = 422.24 $[M + H]^+$.

*5-Methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-2-(pyridin-3-ylmethoxy)benzaldehyde (**43**).* According to the general procedure B, **14a** (571 mg, 2.90 mmol) and **22** (740 mg, 2.42 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (PE/acetone = 3:1) to give **43** (386 mg, 38% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.23 (s, 1H), 8.65 (s, 1H), 8.59 (d, J = 4.4 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.60 (s, 1H), 7.44 (t, J = 7.4 Hz, 2H), 7.40−7.35 (m, 1H), 7.34−7.27 (m, 4H), 7.27−7.25 (m, 2H), 6.14 (s, 1H), 5.20−5.08 (m, 2H), 4.43 (s, 2H), 4.40 (br, 1H), 2.25 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.2, 162.0, 152.8, 149.6, 148.7, 143.3, 141.7, 135.8, 135.1, 133.8, 132.1, 130.0, 129.8, 129.3, 128.2, 127.5, 127.0, 125.9, 123.7, 115.2, 114.9, 93.2, 68.0, 46.7, 16.5, 16.3. ESI-MS: m/z = 423.21 $[M + H]^+$.

*3-((2-Formyl-4-methyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)phenoxy)methyl)benzonitrile (**44**).* According to the general procedure B, **14a** (548 mg, 2.78 mmol) and **20** (766 mg, 2.32 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (PE/acetone = 5:1) to give **44** (382 mg, 37% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.25 (s, 1H), 7.68 (s, 1H), 7.67−7.58 (m, 3H), 7.48 (t, J = 7.6 Hz, 1H), 7.46−7.41 (m, 2H), 7.39−7.35 (m, 1H), 7.34−7.28 (m, 2H), 7.28−7.23 (m, 3H), 6.08 (s, 1H), 5.14 (s, 2H), 4.46 (br, 1H), 4.41 (d, J = 4.8 Hz, 2H), 2.24 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.2, 161.8, 152.8, 143.4, 141.7, 138.2, 135.7, 133.8, 131.8, 131.4, 130.5, 130.2, 130.0, 129.6, 129.4, 128.3, 127.5, 127.1, 126.0, 118.6, 115.3, 115.0, 112.9, 93.3, 69.2, 46.7, 16.5, 16.3. ESI-MS: m/z = 447.21 $[M + H]^+$.

*2-Ethoxy-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)benzaldehyde (**45**).* According to the general procedure B, **14a** (591 mg, 3.0 mmol) and **21** (608 mg, 2.50 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (PE/EA = 7:1) to give **45** (350 mg, 39% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.22 (s, 1H), 7.58 (d, J = 0.8 Hz, 1H), 7.46−7.40 (m, 2H), 7.40−7.34 (m, 1H), 7.34−7.27 (m, 3H), 7.26 (d, J = 3.6 Hz, 1H), 7.24 (s, 1H), 6.09 (s, 1H), 4.44 (d, J = 4.0 Hz, 2H), 4.39 (br, 1H), 4.09 (q, J = 6.8 Hz, 2H), 2.26 (s, 3H), 2.09 (s, 3H), 1.43 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.7, 162.9, 152.8, 143.3, 141.8, 136.0, 133.9, 129.9, 129.6, 129.4, 128.2, 127.8, 127.0, 125.9, 115.1, 114.1, 92.7, 64.1, 46.7, 16.5, 16.3, 14.7. ESI-MS: m/z = 360.21 $[M + H]^+$.

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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*5-((5-((3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)amino)-2-formyl-4-methylphenoxy)methyl)nicotinonitrile (**46**).* According to the general procedure B, **14b** (420 mg, 1.64 mmol) and **19** (654 mg, 1.97 mmol) were used and stirred for 24 h at 85 °C. The crude product was purified on a silica gel column (PE/EA = 1:1) to give **46** (325 mg, 39% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.06 (s, 1H), 8.92 (d, J = 2.0 Hz, 1H), 8.83 (d, J = 2.4 Hz, 1H), 8.33 (t, J = 2.0 Hz, 1H), 7.38 (d, J = 0.8 Hz, 1H), 7.13−7.08 (m, 2H), 7.07−7.05 (m, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 6.77 (dd, J = 8.2, 2.2 Hz, 1H), 6.67 (t, J = 5.8 Hz, 1H), 6.09 (s, 1H), 5.21 (s, 2H), 4.47 (d, J = 5.6 Hz, 2H), 4.29 (s, 4H), 2.23 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.6, 161.1, 153.5, 151.9, 151.7, 142.9, 142.4, 141.5, 138.4, 136.9, 134.7, 133.1, 132.9, 129.1, 128.4, 125.5, 125.2, 122.2, 117.8, 116.8, 116.7, 115.2, 113.6, 108.9, 93.3, 66.3, 64.1, 44.8, 16.9, 16.0. ESI-MS: m/z = 506.20 $[M + H]^+$.

*5-((4-Chloro-5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)amino)-2-formylphenoxy)methyl)nicotinonitrile (**47**).* According to the general procedure B, **14b** (418 mg, 1.64 mmol) and **24** (480 mg, 1.37 mmol) were used and stirred for 24 h at reflux. The crude product was purified on a silica gel column (PE/EA = 3:2) to give **47** (559 mg, 78% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.04 (s, 1H), 8.93 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.36 (t, J = 2.0 Hz, 1H), 7.60 (s, 1H), 7.14−7.03 (m, 4H), 6.92 (d, J = 8.2 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 8.2, 2.2 Hz, 1H), 6.26 (s, 1H), 5.25 (s, 2H), 4.56 (d, J = 6.0 Hz, 2H), 4.29 (s, 4H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.3, 160.8, 151.9, 151.8, 150.2, 142.9, 142.4, 141.6, 138.5, 136.3, 134.6, 132.8, 132.6, 128.5, 128.4, 125.3, 125.1, 122.1, 117.8, 116.7, 116.7, 114.4, 111.3, 108.9, 95.2, 66.6, 64.1, 44.6, 15.9. ESI-MS: m/z = 526.19 $[M + H]^+$.

General Procedure C for Reductive Amination Reaction. Aldehydes (1 equiv), amines (2 equiv), and AcOH (2 equiv) were suspended in an appropriate solvent and stirred for 24 h at 35 °C. After addition of $NaBH(OAc)_3$ (3 equiv), the mixture was stirred for further 24 h. Then, the mixture was diluted with water and extracted with DCM/MeOH (10:1). The combined organic phase was washed with water and concentrated to dryness in vacuo. The crude product was purified by preparative high-performance liquid chromatography.

*N-(2-(((2-Methoxy-6-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)pyridin-3-yl)methyl)amino)ethyl)acetamide(**48**).* According to a similar manner as the general procedure C, **38** (157 mg, 0.47 mmol) was reacted with *N*-(2-amino-ethyl)acetamide (97 mg, 0.94 mmol) in DCE (4 mL) and DMF (1 mL). After completion of $NaBH(OAc)_3$ addition, the mixture was stirred for further 12 h. The reaction yielded **48** (85 mg, 43% yield) as a yellowish solid. mp 119.2−120.3 °C. HPLC purity: 96.00% (method B). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (s, 1H), 7.47−7.39 (m, 3H), 7.39−7.32 (m, 1H), 7.32−7.27 (m, 3H), 7.24−7.16 (m, 2H), 6.54 (br, 1H), 5.94 (d, J = 8.0 Hz, 1H), 4.79 (t, J = 5.2 Hz, 1H), 4.46 (d, J = 5.2 Hz, 2H), 3.93 (s, 3H), 3.88 (s, 2H), 3.54−3.47 (m, 2H), 2.95 (t, J = 4.8 Hz, 2H), 2.22 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 161.6, 158.3, 143.0, 142.2, 142.1, 137.0, 133.8, 129.5, 129.4, 128.1, 127.5, 126.9, 125.7, 100.2, 97.9, 53.4, 47.0, 46.5, 45.0, 36.2, 23.2, 16.3. ESI-HRMS: m/z calculated for $C_{25}H_{30}N_4NaO_2^+$ $[M + Na]^+$, 441.2261; found, 441.2259.

*N-(2-((2-((3-Cyanobenzyl)oxy)-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)amino)ethyl)acetamide (**49**).* According to a similar manner as the general procedure C, **30** (100 mg, 0.23 mmol) was reacted with *N*-(2-aminoethyl)acetamide (47 mg, 0.46 mmol) in DCE (2 mL) and DMF (0.5 mL). After completion of $NaBH(OAc)_3$ addition, the mixture was stirred for further 14 h, diluted with water, and extracted with DCM. The combined organic phase was washed with water and concentrated to dryness in vacuo. The crude product was purified by silica gel column chromatography (DCM/MeOH = 10:1) and recrystallization (EA/*n*-hexane) to give **49** (42 mg, 35% yield) as a pale-yellow solid. mp 147.7−148.7 °C. HPLC purity: 95.99% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.90 (s, 1H), 7.80−7.76 (m, 3H), 7.60 (t, J = 7.6 Hz, 1H), 7.45 (t, J = 7.6 Hz, 2H), 7.39−7.34 (m, 1H), 7.31−7.28 (m, 3H), 7.19 (t, J = 7.6 Hz, 1H), 7.10 (dd, J = 7.5, 1.1 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.35 (d, J = 2.0 Hz, 1H), 6.15 (dd, J = 8.0, 2.0 Hz, 1H), 6.03 (t, J = 5.6 Hz, 1H), 5.10 (s, 2H), 4.23 (d, J = 5.6 Hz, 2H), 3.57 (s, 2H), 3.11 (q, J = 6.4 Hz, 2H), 2.52−2.50 (m, 2H), 2.18 (s, 3H), 1.81 (br, 1H), 1.76 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.0, 156.6, 149.2, 141.9, 141.7, 139.4, 138.2, 133.1, 131.9, 131.4, 130.6, 130.2, 129.7, 129.2, 128.3, 128.2, 126.9, 126.8, 125.3, 118.8, 116.2, 111.4, 103.9, 97.3, 67.8, 48.2, 47.5, 45.6, 38.8, 22.6, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{35}N_4O_2^+$ $[M + H]^+$, 519.2755; found, 519.2754.

*3-((2-((((2-Hydroxyethyl)amino)methyl)-5-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)phenoxy)methyl)benzonitrile (**50**).* According to a similar manner as the general procedure C, **30** (108 mg, 0.25 mmol) was reacted with 2-aminoethanol (31 mg, 0.5 mmol) in DCE (3 mL) and stirred for 12 h at rt. After completion of $NaBH(OAc)_3$ addition, the mixture was stirred for further 12 h. The crude product was purified by silica gel column chromatography (DCM/MeOH = 10:1−8:1) and recrystallization (EA/*n*-hexane) to give **50** (30 mg, 25% yield) as a pale-yellow solid. mp 173.1−174.2 °C. HPLC purity: 98.56% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.90 (s, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.59 (t, J = 8.0 Hz, 1H), 7.45 (t, J = 7.2 Hz, 2H), 7.38−7.35 (m, 1H), 7.31−7.29 (m, 3H), 7.19 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 7.2 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.36 (s, 1H), 6.15 (d, J = 7.8 Hz, 1H), 6.03 (t, J = 5.6 Hz, 1H), 5.10 (s, 2H), 4.46 (br, 1H), 4.23 (d, J = 4.8 Hz, 2H), 3.58 (s, 2H), 3.44 (t, J = 5.2 Hz, 2H), 2.53 (t, J = 6.0, 2H), 2.18 (s, 3H), 1.90 (br, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.7, 149.1, 141.9, 141.7, 139.4, 138.2, 133.1, 132.0, 131.4, 130.6, 130.2, 129.7, 129.2, 128.3, 128.2, 126.9, 126.8, 125.3, 118.7, 116.3, 115.4, 111.3, 103.8, 97.3, 67.8, 60.5, 51.1, 47.9, 45.6, 15.8. ESI-HRMS: m/z calculated for $C_{31}H_{31}N_3NaO_2^+$ $[M + Na]^+$, 500.2308; found, 500.2307.

*3-((2-((((1,3-Dihydroxypropan-2-yl)amino)methyl)-5-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)phenoxy)methyl)benzonitrile (**51**).* According to a similar manner as the general procedure C, **30** (108 mg, 0.25 mmol) was reacted with 2-amino-1,3-propanediol (46 mg, 0.5 mmol) in DCE (2 mL) and DMF (0.5 mL). The crude product was purified by recrystallization (EA/*n*-hexane) to give **51** (92 mg, 72% yield) as a pale-yellow solid. mp 137.6−138.5 °C. HPLC purity: 97.72% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.92 (s, 1H), 7.82−7.78 (m, 2H), 7.58 (t, J = 8.0 Hz, 1H), 7.48−7.42 (m, 2H), 7.40−7.34 (m, 1H), 7.32−7.27 (m, 3H), 7.19 (t, J = 7.6 Hz, 1H), 7.10 (dd, J = 7.6, 1.2 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 6.35 (d, J = 2.0 Hz, 1H), 6.15 (dd, J = 8.0, 1.6 Hz, 1H), 6.02 (t, J = 5.2 Hz, 1H), 5.10 (s, 2H), 4.38 (br, 1H), 4.23 (d, J = 5.6 Hz, 2H), 3.62 (s, 2H), 3.36 (ddd, J = 26.2, 10.8, 5.6 Hz, 4H), 2.55 (p, J = 5.6 Hz, 1H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.2, 149.7, 142.4, 142.2, 139.8, 138.7, 133.6, 132.5, 131.9, 131.2, 130.8, 130.1, 129.7, 128.8, 128.7, 127.4, 127.3, 125.8, 119.3, 117.0, 111.8, 104.3, 97.8, 68.3, 61.7, 60.6, 46.3, 46.1, 16.3. ESI-HRMS: m/z calculated for $C_{32}H_{33}N_3NaO_3^+$ $[M + Na]^+$, 530.2414; found, 530.2413.

*(2-((3-Cyanobenzyl)oxy)-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**52**).* According to the general procedure C, **30** (216 mg, 0.50 mmol) was reacted with D-serine (105 mg, 1.0 mmol) in DMF (2 mL), which yielded **52** (180 mg, 69% yield) as a yellowish solid. mp 173.5−174.4 °C. HPLC purity: 98.15% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.31 (br, 2H), 7.99 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.57 (t, J = 8.0 Hz, 1H), 7.47−7.44 (m, 2H), 7.40−7.35 (m, 1H), 7.31 (d, J = 1.2 Hz, 1H), 7.29 (s, 1H), 7.26 (d, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.40−6.35 (m, 2H), 6.19 (dd, J = 8.0, 1.6 Hz, 1H), 5.41 (br, 1H), 5.18−5.11 (m, 2H), 4.25 (d, J = 5.2 Hz, 2H), 4.04 (dd, J = 43.6, 12.8 Hz, 2H), 3.78 (dd, J = 11.6, 4.0 Hz, 1H), 3.66 (dd, J = 11.2, 7.2 Hz, 1H), 3.19−3.16 (m, 1H), 2.17 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.1, 157.3, 151.2, 142.0, 141.7, 138.8, 137.9, 133.2, 132.4, 132.3, 131.6, 130.9, 129.6, 129.2, 128.4, 128.2, 126.8, 125.3, 118.8, 111.4, 107.4, 104.0, 96.7, 68.2, 61.9, 59.9, 45.6, 45.4, 15.8. ESI-HRMS: m/z calculated for $C_{32}H_{31}N_3NaO_4^+$ $[M + Na]^+$, 544.2207; found, 544.2208.

*(2-((3-Cyanobenzyl)oxy)-6-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**53**).* According to the general

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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procedure C, **36** (131 mg, 0.29 mmol) was reacted with D-serine (62 mg, 0.58 mmol) in anhydrous DMF (4 mL) and DCE (1 mL), which yielded **53** (48 mg, 31% yield) as a white solid. mp 167.5−169.0 °C. HPLC purity: 97.97% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (br, 2H), 7.98 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.56 (dt, J = 7.6, 1.2 Hz, 1H), 7.49−7.41 (m, 2H), 7.40−7.34 (m, 1H), 7.32−7.28 (m, 2H), 7.27−7.23 (m, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.10 (dd, J = 7.6, 1.2 Hz, 1H), 6.29 (t, J = 5.2 Hz, 1H), 6.24 (d, J = 1.6 Hz, 1H), 6.12 (d, J = 1.6 Hz, 1H), 5.37 (br, 1H), 5.13 (dd, J = 14.8, 13.2 Hz, 2H), 4.24 (d, J = 5.6 Hz, 2H), 4.10 (s, 2H), 3.84 (dd, J = 11.6, 4.4 Hz, 1H), 3.61 (dd, J = 11.6, 8.4 Hz, 1H), 3.19 (dd, J = 8.0, 4.4 Hz, 1H), 2.21 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.6, 157.8, 150.4, 142.0, 141.7, 139.5, 138.7, 137.9, 133.2, 132.4, 131.6, 130.9, 129.7, 129.2, 128.4, 128.3, 126.9, 125.3, 118.8, 115.5, 111.4, 106.2, 94.5, 68.2, 62.5, 59.9, 45.2, 42.7, 19.6, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{33}N_3NaO_4^+$ [M + Na]$^+$, 558.2363; found, 558.2363.

*(2-((3-Cyanobenzyl)oxy)-6-methoxy-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**54**).* According to the general procedure C, **31** (189 mg, 0.41 mmol) was reacted with D-serine (86 mg, 0.82 mmol) in anhydrous DMF (4 mL), which yielded **54** (136 mg, 60% yield) as a white solid. mp 157.2−158.5 °C. HPLC purity: 98.43% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (br, 2H), 7.95 (s, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.48−7.43 (m, 2H), 7.40−7.34 (m, 1H), 7.31−7.28 (m, 3H), 7.20 (t, J = 7.6 Hz, 1H), 7.12 (dd, J = 7.2, 1.2 Hz, 1H), 6.42 (t, J = 5.2 Hz, 1H), 6.02 (d, J = 3.2 Hz, 2H), 5.31 (br, 1H), 5.13 (dd, J = 16.4, 13.2 Hz, 2H), 4.28 (d, J = 5.2 Hz, 2H), 4.09 (dd, J = 26.8, 13.2 Hz, 2H), 3.78 (dd, J = 11.2, 4.4 Hz, 1H), 3.73 (s, 3H), 3.62 (dd, J = 11.6, 7.6 Hz, 1H), 3.12 (dd, J = 7.2, 4.4 Hz, 1H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 159.5, 158.1, 151.5, 142.1, 141.6, 138.7, 137.8, 133.3, 132.2, 131.5, 130.8, 129.6, 129.2, 128.5, 128.2, 127.2, 126.8, 125.4, 118.7, 111.4, 95.3, 89.3, 88.7, 68.3, 62.1, 59.6, 55.5, 45.4, 15.9. ESI-HRMS: m/z calculated for $C_{33}H_{33}N_3NaO_5^+$ [M + Na]$^+$, 574.2312; found, 574.2313.

*(2-((3-Cyanobenzyl)oxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**55**).* According to the general procedure C, **44** (341 mg, 0.76 mmol) was reacted with D-serine (161 mg, 1.53 mmol) in anhydrous DMF (7 mL), which yielded **55** (320 mg, 79% yield) as a white solid. mp 177.4−178.6 °C. HPLC purity: 97.14% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29 (br, 1H), 7.87 (s, 1H), 7.74−7.68 (m, 2H), 7.47−7.43 (m, 3H), 7.39−7.35 (m, 1H), 7.33−7.28 (m, 2H), 7.17 (dd, J = 7.2, 1.2 Hz, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.08 (dd, J = 7.2, 1.6 Hz, 1H), 6.99 (s, 1H), 6.09 (s, 1H), 5.70 (t, J = 5.6 Hz, 1H), 5.38 (br, 1H), 5.12−5.02 (m, 2H), 4.33 (d, J = 5.2 Hz, 2H), 4.02 (dd, J = 40.4, 13.0 Hz, 2H), 3.79 (dd, J = 11.6, 4.4 Hz, 1H), 3.65 (dd, J = 11.2, 7.2 Hz, 1H), 3.18 (dd, J = 6.9, 4.6 Hz, 1H), 2.19 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.8, 155.8, 148.2, 141.9, 141.7, 138.9, 137.8, 132.80, 132.7, 132.0, 131.4, 130.6, 129.5, 129.2, 128.2, 126.8, 126.0, 125.3, 118.7, 113.8, 111.4, 106.7, 95.0, 68.4, 61.9, 59.9, 45.5, 45.1, 16.9, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{33}N_3NaO_4^+$ [M + Na]$^+$, 558.2363; found, 558.2364.

*(2-(Benzyloxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)-methyl)amino)benzyl)-D-serine (**56**).* According to the general procedure C, **42** (297 mg, 0.70 mmol) was reacted with D-serine (148 mg, 1.41 mmol) in anhydrous DMF (8 mL) and anhydrous MeOH (2 mL), which yielded **56** (36 mg, 10% yield) as a white solid. mp 186.6−187.8 °C. HPLC purity: 98.26% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (br, 2H), 7.46 (tt, J = 8.2, 1.6 Hz, 2H), 7.41−7.37 (m, 1H), 7.35−7.32 (m, 3H), 7.31 (t, J = 1.2 Hz, 1H), 7.30−7.24 (m, 3H), 7.21 (dd, J = 7.6, 1.6 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.10 (dd, J = 7.4, 1.4 Hz, 1H), 6.97 (s, 1H), 6.13 (s, 1H), 5.70 (t, J = 5.6 Hz, 1H), 5.38 (br, 1H), 4.98 (s, 2H), 4.32 (d, J = 5.2 Hz, 2H), 3.99 (dd, J = 36.2, 13.0 Hz, 2H), 3.78 (dd, J = 11.4, 4.4 Hz, 1H), 3.66 (dd, J = 11.4, 7.0 Hz, 1H), 3.17 (dd, J = 7.2, 4.4 Hz, 1H), 2.20 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.0, 156.0, 148.1, 141.9, 141.7, 137.9, 137.1, 132.7, 132.6, 129.2, 128.3, 128.2, 128.1, 127.6, 127.4, 126.8, 126.0, 125.3, 113.6, 106.7, 95.3, 69.6, 61.8, 59.8, 45.5, 45.1, 16.8, 15.8. ESI-HRMS: m/z calculated for $C_{32}H_{34}N_2NaO_4^+$ [M + Na]$^+$, 533.2411; found, 533.2408.

*(5-Methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-2-(pyridin-3-ylmethoxy)benzyl)-D-serine (**57**).* According to the general procedure C, **43** (310 mg, 0.73 mmol) was reacted with D-serine (154 mg, 1.47 mmol) in anhydrous DMF (8 mL) and anhydrous MeOH (2 mL), which yielded **57** (51 mg, 14% yield) as a white solid. mp 196.4−197.9 °C. HPLC purity: 99.61% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (d, J = 1.6 Hz, 1H), 8.47 (dd, J = 4.8, 1.6 Hz, 1H), 8.26 (br, 2H), 7.81 (dt, J = 8.0, 2.0 Hz, 1H), 7.49−7.42 (m, 2H), 7.40−7.35 (m, 1H), 7.34−7.31 (m, 2H), 7.29 (ddd, J = 7.8, 4.8, 0.4 Hz, 1H), 7.21 (dd, J = 7.6, 1.2 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.09 (dd, J = 7.4, 1.4 Hz, 1H), 6.98 (s, 1H), 6.15 (s, 1H), 5.69 (t, J = 5.6 Hz, 1H), 5.38 (br, 1H), 5.04 (dd, J = 15.6, 13.2 Hz, 2H), 4.35 (d, J = 5.2 Hz, 2H), 3.99 (dd, J = 40.8, 13.2 Hz, 2H), 3.78 (dd, J = 11.4, 4.4 Hz, 1H), 3.65 (dd, J = 11.2, 7.2 Hz, 1H), 3.17 (dd, J = 7.0, 4.6 Hz, 1H), 2.21 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 155.8, 148.9, 148.7, 148.1, 141.9, 141.7, 137.8, 135.3, 132.8, 132.7, 132.6, 129.2, 128.1, 126.8, 126.1, 125.3, 123.4, 113.8, 106.9, 95.1, 67.3, 61.8, 59.9, 45.5, 45.1, 16.8, 15.8. ESI-HRMS: m/z calculated for $C_{31}H_{33}N_3NaO_4^+$ [M + Na]$^+$, 534.2363; found, 534.2360.

*(2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**58**).* According to the general procedure C, **35** (750 mg, 1.68 mmol) was reacted with D-serine (350 mg, 3.36 mmol) in anhydrous DMF (12 mL) and anhydrous MeOH (4 mL), which yielded **58** (350 mg, 39% yield) as a white solid. mp 172.3−173.9 °C. HPLC purity: 97.93% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (dd, J = 15.6, 2.0 Hz, 2H), 8.37 (t, J = 2.0 Hz, 1H), 8.34 (br, 2H), 7.48−7.44 (m, 2H), 7.40−7.35 (m, 1H), 7.35−7.34 (m, 1H), 7.33−7.32 (m, 1H), 7.16 (dd, J = 7.6, 1.8 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.07 (dd, J = 7.2, 1.8 Hz, 1H), 7.01 (s, 1H), 6.09 (s, 1H), 5.72 (t, J = 5.6 Hz, 1H), 5.38 (br, 1H), 5.14 (dd, J = 18.8, 13.2 Hz, 2H), 4.35 (d, J = 5.6 Hz, 2H), 4.04 (dd, J = 44.8, 12.8 Hz, 2H), 3.78 (dd, J = 11.2, 4.4 Hz, 1H), 3.64 (dd, J = 11.4, 7.2 Hz, 1H), 3.19−3.16 (m, 1H), 2.19 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.9, 155.6, 151.9, 151.6, 148.2, 141.9, 141.7, 138.4, 137.7, 133.4, 133.0, 132.7, 129.3, 128.2, 126.8, 125.9, 125.2, 116.9, 114.0, 108.8, 106.6, 94.8, 66.3, 61.9, 59.9, 45.4, 45.1, 16.9, 15.8. ESI-HRMS: m/z calculated for $C_{32}H_{32}N_4NaO_4^+$ [M + Na]$^+$, 559.2316; found, 559.2313.

*(2-((5-Cyanopyridin-3-yl)methoxy)-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**59**).* According to the general procedure C, **32** (133 mg, 0.31 mmol) was reacted with D-serine (86 mg, 0.82 mmol) in anhydrous DMF (2.5 mL), which yielded **59** (73 mg, 45% yield) as a white solid. mp 153.8−154.6 °C. HPLC purity: 97.20% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (dd, J = 10.4, 2.4 Hz, 2H), 8.51 (t, J = 2.0 Hz, 1H), 7.48−7.44 (m, 2H), 7.39−7.35 (m, 1H), 7.32−7.31 (m, 1H), 7.31−7.29 (m, 1H), 7.28−7.25 (m, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.09 (t, J = 8.0 Hz, 2H), 6.41−7.25 (m, 2H), 6.21 (dd, J = 8.4, 1.6 Hz, 1H), 5.32 (br, 1H), 5.20 (dd, J = 19.6, 12.8 Hz, 2H), 4.26 (d, J = 5.2 Hz, 2H), 4.03 (dd, J = 46.4, 13.2 Hz, 2H), 3.77 (dd, J = 11.2, 4.4 Hz, 1H), 3.64 (dd, J = 11.4, 7.2 Hz, 1H), 3.17−3.14 (m, 1H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.9, 157.2, 152.4, 151.7, 151.2, 142.0, 141.7, 138.9, 137.8, 133.2, 133.2, 132.5, 129.2, 128.5, 128.2, 126.9, 126.8, 125.3, 116.9, 108.9, 107.4, 104.1, 96.6, 66.1, 61.9, 59.9, 45.5, 45.3, 15.8. ESI-HRMS: m/z calculated for $C_{31}H_{30}N_4NaO_4^+$ [M + Na]$^+$, 545.2159; found, 545.2157.

*(2-((5-Cyanopyridin-3-yl)methoxy)-6-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**60**).* According to the general procedure C, **37** (269 mg, 0.60 mmol) was reacted with D-serine (126 mg, 1.20 mmol) in anhydrous DMF (8 mL) and DCE (2 mL), which yielded **60** (119 mg, 37% yield) as a white solid. mp 183.5−184.7 °C. HPLC purity: 96.76% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (d, J = 2.4 Hz, 1H), 8.99 (d, J = 2.0 Hz, 1H), 8.49 (t, J = 2.4 Hz, 1H), 8.17 (br, 2H), 7.48−7.42 (m, 2H), 7.41−7.34 (m, 1H), 7.34−7.28 (m, 2H), 7.26 (d, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.10 (dd, J = 7.6, 1.6 Hz, 1H), 6.31 (t, J = 5.6 Hz, 1H), 6.25 (d, J = 1.6 Hz, 1H), 6.14 (d, J = 1.6 Hz, 1H), 5.38 (br,

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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1H), 5.18 (dd, J = 18.0, 13.2 Hz, 2H), 4.25 (d, J = 5.2 Hz, 2H), 4.10 (s, 2H), 3.82 (dd, J = 11.6, 4.4 Hz, 1H), 3.61 (dd, J = 11.2, 8.4 Hz, 1H), 3.19 (dd, J = 8.0, 4.4 Hz, 1H), 2.22 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 157.6, 152.4, 151.7, 150.4, 142.0, 141.7, 139.6, 138.9, 137.9, 133.3, 133.2, 129.2, 128.4, 128.2, 126.8, 125.3, 116.9, 108.9, 106.3, 94.4, 66.2, 62.6, 59.9, 45.2, 42.6, 19.5, 15.8. ESI-HRMS: m/z calculated for $C_{32}H_{32}N_4NaO_4^+$ [M + Na]$^+$, 559.2316; found, 559.2317.

(5-Chloro-2-((5-cyanopyridin-3-yl)methoxy)-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**61**). According to the general procedure C, **40** (436 mg, 0.93 mmol) was reacted with D-serine (196 mg, 1.86 mmol) in anhydrous DMF (6 mL), which yielded **61** (106 mg, 20% yield) as a white solid. mp 180.7−182.0 °C. HPLC purity: 99.63% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (d, J = 1.6 Hz, 1H), 8.86 (d, J = 2.0 Hz, 1H), 8.37 (s, 1H), 8.17 (br, 2H), 7.46 (t, J = 7.2 Hz, 2H), 7.40−7.34 (m, 2H), 7.34−7.29 (m, 2H), 7.16−7.03 (m, 3H), 6.24 (s, 1H), 6.09 (t, J = 5.8 Hz, 1H), 5.34 (br, 1H), 5.16 (dd, J = 18.4, 13.2 Hz, 2H), 4.43 (d, J = 5.6 Hz, 2H), 4.01 (dd, J = 33.6, 13.2 Hz, 2H), 3.75 (dd, J = 11.2, 4.4 Hz, 1H), 3.63 (dd, J = 11.2, 6.8 Hz, 1H), 3.17 (dd, J = 6.6, 4.6 Hz, 1H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.4, 156.2, 151.9, 151.7, 145.5, 142.0, 141.6, 138.5, 137.2, 133.0, 132.7, 131.6, 129.2, 128.3, 128.2, 126.9, 125.6, 125.3, 116.8, 109.3, 109.2, 108.9, 96.3, 66.6, 62.3, 60.1, 44.7, 44.5, 15.8. ESI-HRMS: m/z calculated for $C_{31}H_{29}ClN_4NaO_4^+$ [M + Na]$^+$, 579.1770; found, 579.1766.

((2-Methoxy-6-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-pyridin-3-yl)methyl)-D-serine (**62**). According to the general procedure C, **38** (347 mg, 1.04 mmol) was reacted with D-serine (219 mg, 2.08 mmol) in anhydrous DMF (6 mL), which yielded **62** (154 mg, 36% yield) as a white solid. mp 171.9−173.4 °C. HPLC purity: 98.22% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (br, 2H), 7.46−7.42 (m, 2H), 7.40−7.35 (m, 2H), 7.35−7.30 (m, 1H), 7.31−7.25 (m, 2H), 7.24−7.16 (m, 2H), 7.08 (dd, J = 7.6, 0.8 Hz, 1H), 6.08 (d, J = 8.0 Hz, 1H), 5.46 (br, 1H), 4.50 (d, J = 5.6 Hz, 2H), 3.92 (dd, J = 20.0, 11.2 Hz, 2H), 3.81 (s, 3H), 3.76 (dd, J = 11.6, 4.4 Hz, 1H), 3.64 (dd, J = 11.4, 7.0 Hz, 1H), 3.16 (dd, J = 6.8, 4.4 Hz, 1H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.4, 161.0, 158.1, 141.9, 141.7, 141.5, 138.5, 133.0, 129.2, 128.3, 128.2, 127.1, 126.8, 125.3, 115.5, 99.5, 62.3, 60.0, 52.8, 44.9, 43.2, 16.0. ESI-HRMS: m/z calculated for $C_{24}H_{27}N_3NaO_4^+$ [M + Na]$^+$, 444.1894; found, 444.1892.

((4-Methoxy-6-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)-pyridin-3-yl)methyl)-D-serine (**63**). According to the general procedure C, **39** (203 mg, 0.61 mmol) was reacted with D-serine (160 mg, 1.52 mmol) in anhydrous DMF (4 mL). The reaction mixture was extracted with 1-butanol. The combined organic phase was concentrated to dryness in vacuo directly and purified to give **63** (104 mg, 40% yield) as a white solid. mp 177.6−179.2 °C. HPLC purity: 98.86% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (s, 1H), 7.45 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.2 Hz, 1H), 7.30−7.26 (m, 3H), 7.19 (t, J = 7.4 Hz, 1H), 7.12−7.07 (m, 2H), 6.19 (s, 1H), 5.39 (br, 1H), 4.50 (d, J = 5.2 Hz, 2H), 3.93 (dd, J = 21.2, 13.6 Hz, 2H), 3.81−3.75 (m, 4H), 3.64 (dd, J = 11.4, 7.0 Hz, 1H), 3.18 (dd, J = 6.8, 4.4 Hz, 1H), 2.17 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.5, 164.4, 161.1, 150.0, 141.9, 141.7, 138.6, 132.9, 129.2, 128.2, 126.8, 126.7, 125.3, 115.5, 106.5, 89.4, 62.4, 60.0, 55.2, 43.6, 43.0, 15.8. ESI-HRMS: m/z calculated for $C_{24}H_{28}N_3O_4^+$ [M + H]$^+$, 422.2074; found, 422.2074.

(5-Chloro-2-methoxy-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)benzyl)-D-serine (**64**). According to the general procedure C, **33** (150 mg, 0.41 mmol) was reacted with D-serine (86 mg, 0.82 mmol) in anhydrous DMF (3 mL), which yielded **64** (110 mg, 59% yield) as a yellowish solid. mp 197.5−199.1 °C. HPLC purity: 99.72% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47−7.43 (m, 2H), 7.40−7.34 (m, 1H), 7.30 (s, 2H), 7.29−7.26 (m, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.23 (s, 1H), 6.05 (t, J = 5.6 Hz, 1H), 4.48 (d, J = 5.6 Hz, 2H), 3.93 (dd, J = 21.6, 13.2 Hz, 2H), 3.75 (dd, J = 11.6, 4.8 Hz, 1H), 3.67 (s, 3H), 3.65−3.60 (m, 1H), 3.15 (dd, J = 6.8, 4.4 Hz, 1H), 2.23 (s, 3H). ESI-HRMS: m/z calculated for $C_{25}H_{27}ClN_2NaO_4^+$ [M + Na]$^+$, 477.1552; found, 477.1553.

(2-Methoxy-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)benzyl)-D-serine (**65**). According to the general procedure C, **41** (257 mg, 0.74 mmol) was reacted with D-serine (156 mg, 1.48 mmol) in anhydrous DMF (5 mL), which yielded **65** (159 mg, 49% yield) as a white solid. mp 189.9−190.8 °C. HPLC purity: 98.03% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (br, 2H), 7.47−7.43 (m, 2H), 7.40−7.33 (m, 1H), 7.32−7.26 (m, 3H), 7.19 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 6.94 (s, 1H), 6.10 (s, 1H), 5.68 (t, J = 5.6 Hz, 1H), 5.36 (br, 1H), 4.41 (d, J = 5.2 Hz, 2H), 3.95 (dd, J = 27.2, 13.2 Hz, 2H), 3.77 (dd, J = 11.2, 4.4 Hz, 1H), 3.65 (s, 3H), 3.63−3.58 (m, 1H), 3.13 (dd, J = 7.2, 4.4 Hz, 1H), 2.24 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.0, 157.1, 148.3, 142.0, 141.7, 138.1, 132.9, 132.6, 129.2, 128.2, 126.8, 126.4, 125.3, 113.2106.1, 93.5, 62.0, 59.8, 55.3, 45.6, 45.2, 16.9, 15.9. ESI-HRMS: m/z calculated for $C_{26}H_{30}N_2NaO_4^+$ [M + Na]$^+$, 457.2098; found, 457.2096.

(2-Ethoxy-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)-amino)benzyl)-D-serine (**66**). According to the general procedure C, **45** (296 mg, 0.82 mmol) was reacted with D-serine (173 mg, 1.65 mmol) in anhydrous DMF (5.5 mL), which yielded **66** (290 mg, 79% yield) as a white solid. mp 196.1−197.3 °C. HPLC purity: 97.12% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (br, 2H), 7.47−7.43 (m, 2H), 7.40−7.34 (m, 1H), 7.30−7.26 (m, 3H), 7.18 (t, J = 7.6 Hz, 1H), 7.08 (dd, J = 7.6, 1.2 Hz, 1H), 6.93 (s, 1H), 6.06 (s, 1H), 5.71 (t, J = 5.6 Hz, 1H), 5.41 (br, 1H), 4.40 (d, J = 5.6 Hz, 2H), 4.02−3.84 (m, 4H), 3.78 (dd, J = 11.6, 4.4 Hz, 1H), 3.63 (dd, J = 11.6, 7.6 Hz, 1H), 3.14 (dd, J = 7.2, 4.4 Hz, 1H), 2.23 (s, 3H), 2.09 (s, 3H), 1.24 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 156.2, 148.1, 141.9, 141.7, 138.1, 132.8, 132.5, 129.1, 128.2, 128.1, 126.8, 126.2, 125.2, 113.1, 106.4, 94.4, 63.2, 61.9, 59.8, 45.9, 45.0, 16.8, 15.9, 14.3. ESI-HRMS: m/z calculated for $C_{27}H_{32}N_2NaO_4^+$ [M + Na]$^+$, 471.2254; found, 471.2254.

(2-((3-Cyanobenzyl)oxy)-4-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)amino)benzyl)-D-serine (**67**). According to the general procedure C, **34** (162 mg, 0.33 mmol) was reacted with D-serine (69 mg, 0.66 mmol) in anhydrous DMF (3 mL), which yielded **67** (91 mg, 48% yield) as a yellowish solid. mp 172.3−173.5 °C. HPLC purity: 97.12% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (br, 2H), 7.99 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.57 (t, J = 8.0 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 8.0 Hz, 1H), 6.80−6.67 (m, 2H), 6.36−6.34 (m, 2H), 6.19 (d, J = 8.0 Hz, 1H), 5.36 (br, 1H), 5.18−5.10 (m, 2H), 4.28 (br, 4H), 4.23 (d, J = 4.4 Hz, 2H), 4.03 (dd, J = 43.6, 13.2 Hz, 2H), 3.77 (dd, J = 10.8, 4.0 Hz, 1H), 3.65 (dd, J = 11.2, 8.0 Hz, 1H), 3.17−3.14 (m, 1H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.4, 157.8, 151.6, 143.4, 142.9, 141.9, 139.2, 138.2, 135.2, 133.8, 132.8, 132.1, 131.4, 130.1, 128.9, 127.1, 125.7, 122.6, 119.3, 118.2, 117.2, 116.0, 111.9, 107.8, 104.4, 97.2, 68.6, 64.6, 62.4, 60.3, 46.1, 45.9, 16.3. ESI-HRMS: m/z calculated for $C_{34}H_{33}N_3NaO_6^+$ [M + Na]$^+$, 602.2262; found, 602.2266.

(2-((5-Cyanopyridin-3-yl)methoxy)-4-((3-(2,3-dihydrobenzo[b]-[1,4]dioxin-6-yl)-2-methylbenzyl)amino)-5-methylbenzyl)-D-serine (**68**). According to the general procedure C, **46** (288 mg, 0.57 mmol) was reacted with D-serine (120 mg, 1.14 mmol) in anhydrous DMF (6 mL) and anhydrous MeOH (2 mL), which yielded **68** (98 mg, 29% yield) as a white solid. mp 198.1−199.6 °C. HPLC purity: 97.28% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.36 (t, J = 2.0 Hz, 1H), 8.22 (br, 2H), 7.11 (dd, J = 7.2, 1.6 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 7.03 (dd, J = 7.2, 1.6 Hz, 1H), 7.00 (s, 1H), 6.91 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 6.77 (dd, J = 8.4, 2.0 Hz, 1H), 6.07 (s, 1H), 5.69 (t, J = 5.6 Hz, 1H), 5.31 (br, 1H), 5.13 (dd, J = 18.8, 13.2 Hz, 2H), 4.32 (d, J = 5.2 Hz, 2H), 4.28 (s, 4H), 4.03 (dd, J = 44.0, 13.2 Hz, 2H), 3.77 (dd, J = 11.2, 4.4 Hz, 1H), 3.64 (dd, J = 11.6, 7.2 Hz, 1H), 3.16 (dd, J = 7.2, 4.4 Hz, 1H), 2.20 (s, 3H), 2.10 (s, 3H). ESI-HRMS: m/z calculated for $C_{34}H_{34}N_4NaO_6^+$ [M + Na]$^+$, 617.2371; found, 617.2377.

(5-Chloro-2-((5-cyanopyridin-3-yl)methoxy)-4-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)amino)benzyl)-D-

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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*serine (**69**)*. According to the general procedure C, **47** (340 mg, 0.65 mmol) was reacted with D-serine (135 mg, 1.28 mmol) in anhydrous DMF (6 mL) and anhydrous MeOH (2 mL), which yielded **69** (135 mg, 34% yield) as a white solid. mp 203.2−204.8 °C. HPLC purity: 99.00% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.36 (t, J = 2.0 Hz, 1H), 8.17 (br, 2H), 7.37 (s, 1H), 7.12−7.01 (m, 3H), 6.92 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 8.2, 2.2 Hz, 1H), 6.22 (s, 1H), 6.07 (t, J = 5.8 Hz, 1H), 5.38 (br, 1H), 5.15 (dd, J = 18.0, 13.2 Hz, 2H), 4.40 (d, J = 5.6 Hz, 2H), 4.28 (s, 4H), 4.01 (dd, J = 33.6, 13.2 Hz, 2H), 3.75 (dd, J = 11.2, 4.4 Hz, 1H), 3.64 (dd, J = 11.6, 6.8 Hz, 1H), 3.18 (dd, J = 6.8, 4.4 Hz, 1H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.4, 156.1, 151.9, 151.6, 145.4, 142.9, 142.4, 141.5, 138.5, 137.1, 134.7, 133.0, 132.8, 131.6, 128.3, 125.4, 125.3, 122.2, 117.8, 116.8, 116.7, 109.4, 109.3, 108.9, 96.4, 66.6, 64.1, 62.3, 60.1, 44.8, 44.5, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{31}ClN_4NaO_6^+$ $[M + Na]^+$, 637.1824; found, 637.1825.

*(S)-4-((2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)amino)-3-hydroxybutanoic Acid (**70**)*. According to the general procedure C, **35** (313 mg, 0.7 mmol) was reacted with (*S*)-(+)-4-amino-3-hydroxybutyric acid (167 mg, 1.40 mmol) in anhydrous DMF (6.5 mL) and anhydrous MeOH (2 mL), which yielded **70** (97 mg, 25% yield) as a white solid. mp 134.0−135.4 °C. HPLC purity: 97.39% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.90 (d, J = 2.0 Hz, 1H), 8.83 (d, J = 2.0 Hz, 1H), 8.29 (t, J = 2.2 Hz, 1H), 7.48−7.42 (m, 2H), 7.40−7.34 (m, 1H), 7.34−7.31 (m, 2H), 7.17 (dd, J = 7.6, 1.6 Hz, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.07 (dd, J = 7.4, 1.8 Hz, 1H), 6.96 (s, 1H), 6.08 (s, 1H), 5.57 (t, J = 5.6 Hz, 1H), 5.11 (s, 2H), 4.33 (d, J = 5.2 Hz, 2H), 3.88 (qui, J = 5.6 Hz, 1H), 3.76 (dd, J = 17.0, 13.0 Hz, 2H), 2.68 (d, J = 5.6 Hz, 2H), 2.29 (ddd, J = 32.2, 15.2, 5.6 Hz, 2H), 2.20 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.7, 155.3, 151.9, 151.5, 147.4, 141.9, 141.7, 138.3, 137.9, 133.8, 132.7, 132.4, 129.3, 128.2, 126.8, 126.0, 125.2, 121.8, 116.9, 113.9, 110.1, 108.8, 95.2, 66.3, 64.7, 52.9, 45.9, 45.2, 43.5, 17.0, 15.8. ESI-HRMS: m/z calculated for $C_{29}H_{26}N_3O^+$ $[M - C_4H_8NO_3]^+$, 432.2070; found, 432.2069.

*(S)-5-((2-((4-Hydroxy-2-oxopyrrolidin-1-yl)methyl)-4-methyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)phenoxy)methyl)-nicotinonitrile (**71**)*. **71** (38 mg, 10% yield) was obtained from the synthesis of **70** as a white solid. mp 121.6−122.3 °C. HPLC purity: 99.72% (method A). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (d, J = 1.6 Hz, 2H), 8.08 (t, J = 2.0 Hz, 1H), 7.46−7.41 (m, 2H), 7.39−7.34 (m, 1H), 7.34−7.32 (m, 1H), 7.32−7.31 (m, 1H), 7.30−7.27 (m, 1H), 7.24 (d, J = 1.6 Hz, 1H), 7.23 (s, 1H), 6.96 (s, 1H), 6.15 (s, 1H), 5.09 (s, 2H), 4.55 (d, J = 14.4 Hz, 1H), 4.45 (br, 1H), 4.34 (d, J = 14.4 Hz, 1H), 4.32 (s, 2H), 3.86 (br, 1H), 3.50 (dd, J = 10.8, 5.6 Hz, 1H), 3.20 (dd, J = 10.8, 2.0 Hz, 1H), 2.69 (dd, J = 17.2, 6.8 Hz, 1H), 2.44 (br, 1H), 2.39 (dd, J = 17.4, 2.2 Hz, 1H), 2.25 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 155.5, 151.7, 151.7, 147.0, 143.2, 142.0, 138.2, 136.9, 133.8, 133.7, 132.5, 129.6, 129.4, 128.2, 127.5, 127.0, 125.8, 116.5, 115.2, 112.4, 110.1, 95.4, 67.0, 64.5, 55.8, 47.2, 41.5, 40.9, 16.8, 16.3. ESI-HRMS: m/z calculated for $C_{33}H_{33}N_4O_3^+$ $[M + H]^+$, 533.2547; found, 533.2545.

*(2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-homoserine (**72**)*. According to the general procedure C, **35** (313 mg, 0.7 mmol) was reacted with D-homocitrulline (167 mg, 1.40 mmol) in anhydrous DMF (6 mL) and anhydrous MeOH (2 mL), which yielded **72** (230 mg, 60% yield) as a white solid. mp 193.7−194.8 °C. HPLC purity: 99.84% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.90 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.35 (t, J = 2.0 Hz, 1H), 8.15 (br, 2H), 7.48−7.42 (m, 2H), 7.40−7.36 (m, 1H), 7.35−7.31 (m, 2H), 7.17−7.10 (m, 2H), 7.07 (dd, J = 6.8, 2.0 Hz, 1H), 7.01 (s, 1H), 6.08 (s, 1H), 5.73 (t, J = 5.6 Hz, 1H), 5.54 (br, 1H), 5.13 (dd, J = 18.0, 13.2 Hz, 2H), 4.35 (d, J = 5.6 Hz, 2H), 4.03−3.89 (m, 2H), 3.56−3.44 (m, 2H), 3.21 (t, J = 6.4 Hz, 1H), 2.19 (s, 3H), 2.11 (s, 3H), 1.93−1.67 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 155.5, 151.9, 151.5, 148.2, 141.8, 141.7, 138.4, 137.7, 133.4, 132.8, 132.7, 129.2, 128.1, 126.8, 125.9, 125.1, 116.8, 113.9, 109.4, 108.8, 107.0, 94.8, 66.2, 59.9, 59.2, 45.0, 44.9, 32.1, 16.9, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{34}N_4NaO_4^+$ $[M + Na]^+$, 573.2472; found, 573.2472.

*(R)-3-((2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)amino)-4-hydroxybutanoic Acid (**73**)*. According to the general procedure C, **35** (313 mg, 0.7 mmol) was reacted with (*R*)-3-amino-4-hydroxybutanoic acid (167 mg, 1.40 mmol) in anhydrous DMF (6 mL) and anhydrous MeOH (2 mL), which yielded **73** (30 mg, 8% yield) as a white solid. mp 157.9−159.5 °C. HPLC purity: 98.08% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.00 (br, 2H), 8.91−8.80 (m, 2H), 8.32 (s, 1H), 7.45 (t, J = 7.4 Hz, 2H), 7.39−7.33 (m, 3H), 7.16−7.04 (m, 3H), 6.97 (s, 1H), 6.08 (s, 1H), 5.66 (t, J = 5.4 Hz, 1H), 5.17−5.07 (m, 2H), 4.34 (d, J = 4.8 Hz, 2H), 3.91 (dd, J = 68.0, 12.8 Hz, 2H), 3.58 (dd, J = 11.6, 4.4 Hz, 1H), 3.47−3.39 (m, 1H), 3.07−3.01 (m, 1H), 2.19 (s, 3H), 2.16−2.13 (m, 2H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.3, 155.5, 152.0, 151.5, 147.8, 141.9, 141.7, 138.4, 137.8, 133.6, 132.7, 132.5, 129.3, 128.2, 126.8, 125.9, 125.2, 116.9, 113.8, 108.8, 108.8, 95.0, 66.2, 60.3, 55.3, 45.1, 42.9, 33.6, 16.9, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{34}N_4NaO_4^+$ $[M + Na]^+$, 573.2472; found, 573.2472.

*(2S,4R)-1-(2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-4-hydroxypyrrolidine-2-carboxylic Acid (**74**)*. According to the general procedure C, **35** (339 mg, 0.76 mmol) was reacted with L-hydroxyproline (199 mg, 1.52 mmol) in anhydrous DMF (6.5 mL), which yielded **74** (150 mg, 35% yield) as a white solid. mp 171.2−172.9 °C. HPLC purity: 95.54% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.36 (t, J = 2.2 Hz, 1H), 7.47−7.43 (m, 2H), 7.40−7.35 (m, 1H), 7.34−7.32 (m, 1H), 7.32−7.31 (m, 1H), 7.20 (dd, J = 7.6, 1.6 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 7.08 (dd, J = 7.6, 1.6 Hz, 1H), 7.01 (s, 1H), 6.11 (s, 1H), 5.71 (t, J = 5.8 Hz, 1H), 5.36 (br, 1H), 5.15 (dd, J = 23.2, 13.6 Hz, 2H), 4.35 (d, J = 5.6 Hz, 2H), 4.28−4.24 (m, 1H), 4.13 (dd, J = 63.0, 13.0 Hz, 2H), 3.71 (t, J = 8.2 Hz, 1H), 3.33 (dd, J = 11.6, 4.8 Hz, 1H), 2.86 (dd, J = 11.6, 3.2 Hz, 1H), 2.20 (s, 3H), 2.11 (s, 3H), 2.08−1.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.4, 155.7, 151.9, 151.6, 148.3, 141.9, 141.7, 138.5, 137.7, 133.5, 133.4, 132.8, 129.3, 128.2, 128.2, 126.8, 126.1, 125.2, 116.9, 114.2, 108.9, 106.8, 94.9, 68.9, 66.4, 66.4, 59.1, 53.8, 45.2, 37.9, 17.0, 15.9. ESI-HRMS: m/z calculated for $C_{34}H_{34}N_4NaO_4^+$ $[M + Na]^+$, 585.2472; found, 585.2471.

*(2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-asparagine (**75**)*. According to the general procedure C, **35** (313 mg, 0.7 mmol) was reacted with D-asparagine anhydrous (185 mg, 1.40 mmol) in anhydrous DMF (8 mL), which yielded **75** (180 mg, 46% yield) as a white solid. mp 122.3−124.1 °C. HPLC purity: 97.34% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (d, J = 2.0 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.34 (t, J = 2.0 Hz, 1H), 7.78 (s, 1H), 7.49−7.43 (m, 2H), 7.39−7.35 (m, 1H), 7.35−7.34 (m, 1H), 7.33−7.32 (m, 1H), 7.16−7.09 (m, 3H), 7.07 (dd, J = 7.0, 2.2 Hz, 1H), 6.96 (s, 1H), 6.07 (s, 1H), 5.73 (t, J = 5.8 Hz, 1H), 5.16 (s, 2H), 4.34 (d, J = 5.2 Hz, 2H), 4.04 (dd, J = 21.4, 13.0 Hz, 2H), 3.36 (dd, J = 9.2, 4.0 Hz, 1H), 2.73 (dd, J = 16.6, 3.8 Hz, 1H), 2.43 (dd, J = 16.6, 9.0 Hz, 1H), 2.19 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.4, 168.2, 155.5, 151.9, 151.5, 148.3, 141.9, 141.7, 138.3, 137.7, 133.4, 132.8, 132.7, 129.3, 128.2, 126.8, 125.8, 125.2, 116.9, 113.9, 108.8, 106.9, 94.9, 66.2, 57.4, 45.5, 45.1, 34.0, 16.9, 15.8. ESI-HRMS: m/z calculated for $C_{33}H_{33}N_5NaO_4^+$ $[M + Na]^+$, 586.2425; found, 586.2422.

*3-Methyl-2-(trifluoromethyl)-1,1′-biphenyl (**77a**)*. According to the general procedure A, **76a** (10.25 g, 42.88 mmol) and phenylboronic acid (1.4 equiv) were used. The crude product was purified on a silica gel column (PE) to give compound **77a** (5.3 g, 52% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.31 (m, 4H), 7.28−7.25 (m, 3H), 7.12−7.08 (m, 1H), 2.57 (q, J = 2.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.2 (q, J = 2.9 Hz), 141.9, 137.9 (q, J = 2.2 Hz), 133.5, 132.1, 131.7, 130.4, 130.1, 128.5 (q, J = 2.1 Hz), 127.7, 127.2, 126.8, 125.1 (q, J = 274.8 Hz), 21.3 (q, J = 4.0 Hz).

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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*2-Bromo-3-methyl-1,1′-biphenyl (**77b**).* According to the general procedure A, **76b** (5 g, 16.8 mmol) and phenylboronic acid (1.15 equiv) were used. The crude product was purified on a silica gel column (PE) to give compound **77b** (3.93 g, 95% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61−7.58 (m, 1H), 7.48−7.31 (m, 6H), 7.26−7.23 (m, 1H), 7.16−7.10 (m, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.4, 142.2, 138.9, 129.8, 129.5, 128.7, 127.9, 127.5, 126.8, 125.4, 24.4.

*3-(Bromomethyl)-2-(trifluoromethyl)-1,1′-biphenyl (**78a**).* BPO (75% w/w, 32 mg, 0.10 mmol) was added to a solution of **77a** (473 mg, 2.0 mmol) and NBS (356 mg, 2 mmol) in CCl_4 (8 mL) and stirred for 10 h at reflux. Then, the mixture was concentrated to dryness in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was purified on a silica gel column (PE) to give **78a** (390 mg, 62% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61−7.56 (m, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.40−7.36 (m, 3H), 7.27−7.23 (m, 3H), 4.73 (q, J = 1.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.7 (q, J = 2.9 Hz), 141.1, 137.9 (q, J = 2.1 Hz), 132.7, 132.2, 131.2, 128.5 (q, J = 2.0 Hz), 127.9, 127.5, 126.4 (q, J = 28.9 Hz), 124.5 (q, J = 275.5 Hz), 30.4 (q, J = 4.5 Hz).

*2-Bromo-3-(bromomethyl)-1,1′-biphenyl (**78b**).* According to a similar manner as described for the synthesis of **78a**, **77b** (3.93 g, 15.90 mmol) was used to obtain **78b** (3.36 g, 98% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47−7.42 (m, 2H), 7.41−7.39 (m, 2H), 7.38−7.35 (m, 2H), 7.33 (t, J = 7.6 Hz, 1H), 7.25 (dd, J = 7.6, 2.0 Hz, 1H), 4.71 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.4, 141.4, 137.9, 131.5, 130.3, 129.4, 128.1, 127.8, 127.5, 125.0, 34.8.

*2-((2-(Trifluoromethyl)-[1,1′-biphenyl]-3-yl)methyl)isoindoline-1,3-dione (**79**).* BPO (75% w/w, 304 mg, 1.26 mmol) was added to a solution of **77a** (4.48 g, 18.96 mmol) and NBS (3.37 g, 18.96 mmol) in CCl_4 (70 mL) and stirred for 11 h at reflux. Then, the mixture was concentrated to dryness in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated to dryness in vacuo and dissolved in DMF (50 mL). A phthalimide potassium salt (4.05 g, 21.87 mmol) was added to the above solution and stirred for 1.5 h at 80 °C. The reaction mixture was concentrated to dryness in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water, concentrated in vacuo, and purified on a silica gel column (PE/DCM = 1:1) to give **79** (4.55 g, 60% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98−7.91 (m, 2H), 7.82−7.77 (m, 2H), 7.43−7.36 (m, 4H), 7.31−7.27 (m, 2H), 7.19 (d, J = 7.6 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 5.19 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.7 (q, J = 2.9 Hz), 141.2, 135.6, 134.4, 132.1, 131.6, 130.9, 128.5 (q, J = 2.2 Hz), 127.8, 127.4, 126.1 (q, J = 27.5 Hz), 125.9, 124.5 (q, J = 219.0 Hz), 123.7, 39.2 (q, J = 6.5 Hz). ESI-MS: m/z = 382.09 $[M + H]^+$.

*(2-(Trifluoromethyl)-[1,1′-biphenyl]-3-yl)methanamine (**80**).* According to a similar manner as described for the synthesis of **14a**, **79** (450 mg, 1.18 mmol) was used to obtain **80** (290 mg, 98% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, J = 7.6 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.42−7.33 (m, 3H), 7.29−7.25 (m, 2H), 7.19 (d, J = 7.6 Hz, 1H), 4.08 (q, J = 1.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.1, 142.3 (q, J = 3.0 Hz), 141.6, 131.1, 131.1, 129.4, 128.4 (q, J = 2.0 Hz), 127.8, 127.3, 125.8 (q, J = 28.2 Hz), 125.1 (q, J = 275.5 Hz), 44.7 (q, J = 4.1 Hz). ESI-MS: m/z = 252.09 $[M + H]^+$.

*5-((5-Amino-2-formyl-4-methylphenoxy)methyl)nicotinonitrile (**81**).* According to a similar manner as described for the synthesis of compound **16**, 3-methyl-4-nitrobenzaldehyde (1.90 g, 11.52 mmol) was used to obtain 2-hydroxy-5-methyl-4-nitrobenzaldehyde (**81-1**) (942 mg, 45% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 10.33 (s, 1H), 7.69 (s, 1H), 7.52 (s, 1H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.3, 158.7, 152.6, 132.0, 125.4, 122.3, 112.8, 18.2. ESI-MS: m/z = 180.03 $[M - H]^-$.

According to a similar manner as described for the synthesis of compound **17-1**, **81-1** (578 mg, 3.19 mmol) was used in MeCN/DMF (17 mL/3.5 mL) to obtain 5-((2-formyl-4-methyl-5-nitrophenoxy)methyl)nicotinonitrile (**81-2**) (939 mg, 99% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.44 (s, 1H), 9.04 (t, J = 2.0 Hz, 1H), 8.56 (t, J = 2.0 Hz, 1H), 7.91 (s, 1H), 7.82 (s, 1H), 5.47 (s, 2H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.8, 157.9, 152.8, 152.3, 152.0, 138.9, 132.2, 132.1, 127.0, 124.5, 116.8, 109.8, 109.1, 67.4, 17.9. ESI-MS: m/z = 296.07 $[M - H]^-$.

According to a similar manner as described for the synthesis of compound **17**, **81-2** (1.46 g, 4.91 mmol) was used. The filtrate was concentrated to dryness in vacuo, diluted with water, and extracted with DCM/MeOH (20:1). The combined organic phase was concentrated to give **81** (1.30 g, 99% yield) as an orange solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H), 9.02 (d, J = 2.0 Hz, 1H), 8.99 (d, J = 2.0 Hz, 1H), 8.48 (t, J = 2.0 Hz, 1H), 7.33 (s, 1H), 6.32 (s, 1H), 6.19 (s, 2H), 5.22 (s, 2H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 185.5, 160.6, 154.8, 152.3, 151.8, 138.8, 133.1, 130.1, 116.9, 114.1, 113.8, 109.0, 96.0, 66.1, 16.4. ESI-MS: m/z = 268.11 $[M + H]^+$.

*5-((2-Formyl-5-(((2-(trifluoromethyl)-[1,1′-biphenyl]-3-yl)-methyl)amino)phenoxy)methyl)nicotinonitrile (**82**).* According to a similar manner as described for the synthesis of **30**, **78a** (630 mg, 2 mmol), **17** (532 mg, 2.10 mmol), and K_2CO_3 (332 mg, 2.40 mmol) were suspended in MeCN/DMF (10.5 mL/3.5 mL) and stirred for 11 h at 60 °C. The crude product was purified on a silica gel column (DCM/EA = 7:1) to give **82** (120 mg, 12% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.19 (s, 1H), 8.85 (d, J = 2.0 Hz, 2H), 8.05 (t, J = 2.2 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.44−7.36 (m, 3H), 7.30−7.23 (m, 3H), 6.35 (dd, J = 8.4, 1.6 Hz, 1H), 6.06 (d, J = 2.0 Hz, 1H), 5.16 (s, 2H), 5.04 (t, J = 5.8 Hz, 1H), 4.69 (d, J = 4.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.8, 162.1, 154.4, 152.0, 151.6, 142.9 (q, J = 3.1 Hz), 141.0, 137.8, 137.4, 132.7, 132.0, 131.7, 131.2, 128.3 (q, J = 22.5 Hz), 127.9, 127.9, 127.5, 125.9 (q, J = 29.1 Hz), 124.9 (q, J = 275.2 Hz), 116.3, 116.3, 110.2, 106.6, 94.9, 66.6, 45.34 (q, J = 5.3 Hz). ESI-MS: m/z = 488.14 $[M + H]^+$.

*5-((5-(((2-Bromo-[1,1′-biphenyl]-3-yl)methyl)amino)-2-formylphenoxy)methyl)nicotinonitrile (**83**).* According to a similar manner as described for the synthesis of **30**, **78b** (860 mg, 2.64 mmol), **17** (668 mg, 2.64 mmol), and K_2CO_3 (437 mg, 3.16 mmol) were suspended in MeCN/DMF (15 mL/5 mL) and stirred for 11 h at reflux. The crude product was purified on a silica gel column (DCM/EA = 7:1) to give **83** (200 mg, 15% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.06 (s, 1H), 9.00 (d, J = 1.6 Hz, 1H), 8.97 (d, J = 1.6 Hz, 1H), 8.47 (s, 1H), 7.55−7.50 (m, 2H), 7.50−7.44 (m, 2H), 7.43−7.37 (m, 4H), 7.33 (dd, J = 7.4, 1.4 Hz, 1H), 7.28 (dd, J = 7.2, 1.6 Hz, 1H), 6.34 (d, J = 10.4 Hz, 2H), 5.29 (s, 2H), 4.50 (d, J = 5.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.0, 162.3, 155.6, 152.4, 152.0, 143.2, 141.2, 139.0, 138.4, 135.5, 133.2, 130.4, 129.4, 128.3, 128.1, 127.8, 127.7, 123.2, 117.1, 115.7, 114.7, 109.2, 95.0, 66.4, 47.5. ESI-MS: m/z = 498.08 $[M + H]^+$.

*5-((5-(((2-Bromo-[1,1′-biphenyl]-3-yl)methyl)amino)-2-formyl-4-methylphenoxy)methyl)nicotinonitrile (**84**).* According to a similar manner as described for the synthesis of **30**, **78b** (815 mg, 2.50 mmol), **81** (702 mg, 2.63 mmol), and K_2CO_3 (415 mg, 3.0 mmol) were suspended in MeCN/DMF (12 mL/4 mL) and stirred for 7.5 h at reflux. The crude product was purified on a silica gel column (DCM/EA = 10:1−7:1) to give **84** (180 mg, 14% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.20 (s, 1H), 8.82 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 2.0 Hz, 1H), 7.99 (t, J = 2.2 Hz, 1H), 7.62 (d, J = 0.4 Hz, 1H), 7.49−7.42 (m, 3H), 7.42−7.37 (m, 2H), 7.36−7.29 (m, 2H), 7.28−7.24 (m, 1H), 5.98 (s, 1H), 5.13 (s, 2H), 4.90 (t, J = 5.8 Hz, 1H), 4.61 (d, J = 6.0 Hz, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.9, 161.1, 152.4, 151.9, 151.5, 144.1, 141.1, 137.7, 137.2, 132.9, 130.8, 129.4, 128.2, 127.9, 127.7, 127.5, 123.6, 116.3, 115.9, 115.5, 115.4, 110.1, 93.5, 66.9, 48.7, 16.5. ESI-MS: m/z = 512.08 $[M + H]^+$.

*3-((5-(((2-Bromo-[1,1′-biphenyl]-3-yl)methyl)amino)-2-formylphenoxy)methyl)benzonitrile (**85**).* According to a similar manner as described for the synthesis of **30**, **78b** (400 mg, 1.23

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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mmol), **15** (325 mg, 1.29 mmol), and K_2CO_3 (203 mg, 1.47 mmol) were suspended in MeCN/DMF (4.5 mL/2.5 mL) and stirred for 10 h at reflux. The crude product was purified on a silica gel column (DCM/EA = 70:1) to give **85** (155 mg, 25% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.24 (s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.69−7.60 (m, 3H), 7.50 (t, J = 7.6, 1H), 7.48−7.40 (m, 3H), 7.41−7.34 (m, 2H), 7.32−7.26 (m, 3H), 6.35−6.30 (m, 1H), 6.06 (d, J = 2.0 Hz, 1H), 5.12 (s, 2H), 4.99 (br, 1H), 4.55 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.1, 162.6, 154.5, 144.0, 141.2, 138.0, 137.4, 131.8, 131.3, 131.2, 130.6, 130.4, 129.7, 129.4, 128.1, 127.8, 127.8, 127.4, 123.6, 118.6, 116.3, 112.8, 106.4, 95.2, 68.9, 48.6. ESI-MS: m/z = 497.07 $[M + H]^+$.

*5-((2-Formyl-4-methyl-5-(((2-(trifluoromethyl)-[1,1′-biphenyl]-3-yl)methyl)amino)phenoxy)methyl)nicotinonitrile (**86**).* According to the general procedure B, **80** (290 mg, 1.15 mmol) and **19** (459 mg, 1.39 mmol) were used and stirred for 16 h at reflux. The crude product was purified on a silica gel column (PE/EA = 3:2) to give **86** (388 mg, 67% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.21 (s, 1H), 8.82 (d, J = 1.6 Hz, 1H), 8.79 (d, J = 2.0 Hz, 1H), 7.99 (t, J = 2.0 Hz, 1H), 7.63 (s, 1H), 7.51−7.46 (m, 2H), 7.46−7.38 (m, 3H), 7.30−7.27 (m, 3H), 5.96 (s, 1H), 5.10 (s, 2H), 4.88 (br, 1H), 4.73 (s, 2H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 186.9, 161.0, 152.3, 151.9, 151.5, 142.9 (d, J = 3.0 Hz), 141.0, 137.7, 137.3, 132.8, 132.0, 131.3, 130.8, 128.3 (q, J = 2.4 Hz), 128.0, 127.6, 127.6, 125.9 (q, J = 29.0 Hz), 124.9 (q, J = 275.4 Hz), 116.3, 115.9, 115.4, 110.1, 93.2, 66.71, 45.47 (q, J = 5.3 Hz), 16.5. ESI-MS: m/z = 502.15 $[M + H]^+$.

*(4-(((2-Bromo-[1,1′-biphenyl]-3-yl)methyl)amino)-2-((3-cyanobenzyl)oxy)benzyl)-D-serine (**87**).* According to the general procedure C, **85** (152 mg, 0.31 mmol) was reacted with D-serine (64 mg, 0.61 mmol) in anhydrous DMF (3 mL), which yielded **87** (128 mg, 70% yield) as a white solid. mp 169.9−171.0 °C. HPLC purity: 96.70% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (br, 2H), 7.98 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.57 (t, J = 8.0 Hz, 1H), 7.51−7.29 (m, 6H), 7.24 (dd, J = 7.2, 1.6 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.64 (t, J = 6.0 Hz, 1H), 6.34 (d, J = 1.6 Hz, 1H), 6.12 (dd, J = 8.0, 1.6 Hz, 1H), 5.33 (br, 1H), 5.18−5.10 (m, 2H), 4.35 (d, J = 5.6 Hz, 2H), 4.03 (dd, J = 40.4, 12.8 Hz, 2H), 3.78 (dd, J = 11.6, 4.0 Hz, 1H), 3.65 (dd, J = 11.2, 7.2 Hz, 1H), 3.18−3.15 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.0, 157.4, 150.6, 142.8, 141.1, 139.1, 138.7, 132.5, 132.3, 131.6, 130.9, 129.8, 129.7, 129.3, 128.1, 127.7, 127.6, 127.4, 122.8, 118.8, 111.4, 107.9, 103.9, 96.8, 68.2, 62.0, 59.9, 47.6, 45.5. ESI-HRMS: m/z calculated for $C_{28}H_{22}BrN_2O^+$ $[M - C_3H_6NO_3]^+$, 481.0910; found, 481.0911.

*(4-(((2-Bromo-[1,1′-biphenyl]-3-yl)methyl)amino)-2-((5-cyanopyridin-3-yl)methoxy)benzyl)-D-serine (**88**).* According to the general procedure C, **83** (316 mg, 0.63 mmol) was reacted with D-serine (133 mg, 1.27 mmol) in anhydrous DMF (6 mL), which yielded **88** (153 mg, 41% yield) as a white solid. mp 169.9−171.0 °C. HPLC purity: 98.04% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (d, J = 2.0 Hz, 1H), 8.98 (d, J = 2.0 Hz, 1H), 8.50 (t, J = 2.0 Hz, 1H), 8.22 (br, 1H), 7.50−7.44 (m, 2H), 7.44−7.40 (m, 1H), 7.40−7.34 (m, 3H), 7.32 (dd, J = 7.8, 1.8 Hz, 1H), 7.24 (dd, J = 7.2, 2.0 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 6.66 (t, J = 5.8 Hz, 1H), 6.37 (d, J = 1.6 Hz, 1H), 6.14 (dd, J = 8.2, 1.8 Hz, 1H), 5.31 (br, 1H), 5.20 (dd, J = 19.6, 13.2 Hz, 2H), 4.37 (d, J = 6.0 Hz, 2H), 4.04 (dd, J = 44.4, 13.2 Hz, 2H), 3.77 (dd, J = 11.2, 4.4 Hz, 1H), 3.63 (dd, J = 11.6, 7.2 Hz, 1H), 3.16 (dd, J = 7.2, 4.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.8, 157.3, 152.4, 151.7, 150.6, 142.8, 141.1, 139.1, 138.9, 133.2, 132.6, 129.8, 129.2, 128.1, 127.7, 127.6, 127.4, 122.8, 116.9, 108.9, 107.9, 104.0, 96.8, 66.1, 62.0, 59.9, 47.6, 45.5. ESI-HRMS: m/z calculated for $C_{30}H_{27}BrN_4NaO_4^+$ $[M + Na]^+$, 609.1108; found, 609.1111.

*(4-(((2-Bromo-[1,1′-biphenyl]-3-yl)methyl)amino)-2-((5-cyanopyridin-3-yl)methoxy)-5-methylbenzyl)-D-serine (**89**).* According to the general procedure C, **84** (180 mg, 0.35 mmol) was reacted with D-serine (74 mg, 0.70 mmol) in anhydrous DMF (3.5 mL), which yielded **89** (46 mg, 22% yield) as a white solid. mp 169.1−170.7 °C. HPLC purity: 97.52% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (d, J = 1.6 Hz, 1H), 8.83 (d, J = 1.6 Hz, 1H), 8.34 (s, 1H), 8.14 (br, 2H), 7.48−7.39 (m, 5H), 7.30 (t, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 2H), 7.03 (s, 1H), 6.03−5.96 (m, 2H), 5.29 (br, 1H), 5.12 (dd, J = 18.6, 13.4 Hz, 2H), 4.44 (d, J = 5.6 Hz, 2H), 4.03 (dd, J = 40.0, 13.0 Hz, 2H), 3.77 (dd, J = 11.4, 4.2 Hz, 1H), 3.64 (dd, J = 11.0, 7.4 Hz, 1H), 3.17 (dd, J = 6.8, 4.4 Hz, 1H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.8, 155.6, 151.8, 151.6, 147.7, 142.7, 141.1, 139.0, 138.4, 133.3, 133.1, 129.8, 129.3, 128.1, 127.6, 127.3, 122.6, 116.8, 114.0, 108.8, 107.2, 94.7, 66.3, 62.0, 59.9, 47.4, 45.4, 16.9. ESI-HRMS: m/z calculated for $C_{31}H_{29}BrN_4NaO_4^+$ $[M + Na]^+$, 623.1264; found, 623.1265.

*(2-((5-Cyanopyridin-3-yl)methoxy)-5-methyl-4-(((2-(trifluoromethyl)-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**90**).* According to the general procedure C, **86** (358 mg, 0.71 mmol) was reacted with D-serine (150 mg, 1.43 mmol) in anhydrous DMF (6 mL) and anhydrous MeOH (2 mL), which yielded **90** (181 mg, 43% yield) as a white solid. mp 131.5−132.6 °C. HPLC purity: 97.97% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (d, J = 2.0 Hz, 1H), 8.82 (d, J = 2.0 Hz, 1H), 8.34 (t, J = 2.0 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.46−7.38 (m, 4H), 7.31 (d, J = 2.0 Hz, 1H), 7.29 (d, J = 1.6 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H), 7.05 (s, 1H), 6.09 (t, J = 5.6 Hz, 1H), 5.93 (s, 1H), 5.32 (br, 1H), 5.09 (dd, J = 17.8, 13.2 Hz, 2H), 4.57 (d, J = 4.0 Hz, 2H), 4.03 (dd, J = 40.4, 13.2 Hz, 2H), 3.78 (dd, J = 11.4, 4.4 Hz, 1H), 3.64 (dd, J = 11.6, 7.2 Hz, 1H), 3.19−3.16 (m, 1H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.0, 155.6, 151.8, 151.6, 147.7, 141.6, 141.1, 139.6, 138.4, 133.3, 133.2, 131.4, 130.9, 128.2, 127.9, 127.4, 127.1, 125.1 (q, J = 276 Hz), 124.3 (q, J = 28.4 Hz), 116.8, 114.1, 108.8, 107.1, 94.3, 66.2, 62.0, 59.9, 45.3, 44.0, 16.9. ESI-HRMS: m/z calculated for $C_{32}H_{29}F_3N_4NaO_4^+$ $[M + Na]^+$, 613.2033; found, 613.2033.

*(2-((5-Cyanopyridin-3-yl)methoxy)-4-(((2-(trifluoromethyl)-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**91**).* According to the general procedure C, **82** (120 mg, 0.25 mmol) was reacted with D-serine (52 mg, 0.49 mmol) in anhydrous DMF (3 mL), which yielded **91** (56 mg, 39% yield) as a white solid. mp 164.9−166.1 °C. HPLC purity: 97.16% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (d, J = 2.0 Hz, 1H), 8.99 (d, J = 2.0 Hz, 1H), 8.50 (t, J = 2.0 Hz, 1H), 8.21 (br, 2H), 7.60−7.55 (m, 2H), 7.47−7.37 (m, 3H), 7.31−7.26 (m, 2H), 7.22 (t, J = 4.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.72 (t, J = 5.6 Hz, 1H), 6.35 (d, J = 1.6 Hz, 1H), 6.13 (dd, J = 8.0, 1.6 Hz, 1H), 5.34 (br, 1H), 5.19 (dd, J = 19.6, 13.2 Hz, 2H), 4.49 (d, J = 4.0 Hz, 2H), 4.03 (dd, J = 44.0, 13.2 Hz, 2H), 3.76 (dd, J = 11.6, 4.4 Hz, 1H), 3.63 (dd, J = 11.6, 7.2 Hz, 1H), 3.16 (dd, J = 7.2, 4.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.9, 157.3, 152.3, 151.7, 150.6, 141.7, 141.1, 139.5, 138.9, 133.2, 132.7, 131.4, 131.0, 128.2, 127.9, 127.4, 124.9 (q, J = 275.9 Hz), 124.5 (q, J = 28.4 Hz), 116.9, 108.9, 108.0, 103.9, 96.7, 66.1, 62.0, 59.9, 45.4, 44.1. ESI-HRMS: m/z calculated for $C_{31}H_{27}F_3N_4NaO_4^+$ $[M + Na]^+$, 599.1877; found, 599.1876.

*3-((2-Formyl-5-(methyl((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)phenoxy)methyl)benzonitrile (**92**).* Methyl benzenesulfonate (65 mg, 0.38 mmol) was added to a suspension of **30** (108 mg, 0.25 mmol) and KOH (85% w/w, 20 mg, and 0.30 mmol) in THF (3 mL) and stirred for 25 h at 35 °C. Then, a further amount of methyl benzenesulfonate (13 mg, 0.08 mmol) was added to the reaction mixture and stirred for 15 h. The mixture was concentrated to dryness in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated to dryness and purified by recrystallization (PE/DCM) to give compound **92** (70 mg, 63% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.24 (s, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.69 (s, 1H), 7.67−7.59 (m, 2H), 7.51−7.41 (m, 3H), 7.41−7.35 (m, 1H), 7.34−7.29 (m, 2H), 7.23−7.15 (m, 2H), 6.94 (dd, J = 6.4, 2.4 Hz, 1H), 6.38 (dd, J = 8.8, 2.0 Hz, 1H), 6.09 (d, J = 2.4 Hz, 1H), 5.14 (s, 2H), 4.59 (s, 2H), 3.19 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.0, 162.4, 155.6, 143.2, 141.7, 138.1, 134.7, 132.7, 131.8, 131.3, 130.9, 130.4, 129.6, 129.4, 129.2, 128.2, 127.0, 125.9, 124.4, 118.6, 115.3, 112.8, 105.5, 94.7, 68.9, 55.0, 39.0, 16.0. ESI-MS: m/z = 447.22 $[M + H]^+$.

*(2-((3-Cyanobenzyl)oxy)-4-(methyl((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)benzyl)-D-serine (**93**).* According to the general

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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procedure C, **92** (89 mg, 0.2 mmol) was reacted with D-serine (42 mg, 0.40 mmol) in anhydrous DMF (2 mL). After completion of $NaBH(OAc)_3$ addition, the mixture was stirred for further 14 h. The crude product was purified by preparative high-performance liquid chromatography to obtain **94** (64 mg, 60% yield) as a yellowish solid. mp 178.6−179.9 °C. HPLC purity: 97.40% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (br, 2H), 7.98 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 7.41−7.35 (m, 1H), 7.33−7.29 (m, 2H), 7.17−7.11 (m, 2H), 7.08 (d, J = 7.6 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 6.37 (d, J = 2.0 Hz, 1H), 6.25 (dd, J = 8.4, 2.0 Hz, 1H), 5.39 (br, 1H), 5.21 (dd, J = 17.2, 13.2 Hz, 2H), 4.56 (s, 2H), 4.06 (dd, J = 42.4, 12.8 Hz, 2H), 3.78 (dd, J = 11.6, 4.4 Hz, 1H), 3.66 (dd, J = 11.2, 7.2 Hz, 1H), 3.18 (dd, J = 6.8, 4.4 Hz, 1H), 3.04 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.1, 157.4, 151.2, 142.1, 141.5, 138.8, 136.5, 132.6, 132.3, 132.3, 131.5, 130.9, 129.6, 129.2, 128.2, 128.1, 126.9, 125.3, 124.5, 118.7, 111.4, 107.6, 104.1, 96.3, 68.3, 62.0, 59.9, 53.8, 45.4, 38.4, 15.5. ESI-HRMS: m/z calculated for $C_{33}H_{33}N_3NaO_4^+$ [M + Na]$^+$, 558.2363; found, 558.2361.

*3-((2-(((2-Hydroxyethyl)amino)methyl)-5-(methyl((2-methyl-[1,1′-biphenyl]-3-yl)methyl)amino)phenoxy)methyl)benzonitrile (**94**).* According to the general procedure C, **92** (70 mg, 0.16 mmol) was reacted with 2-aminoethanol (19 mg, 0.31 mmol) in DCE (2 mL) and anhydrous DMF (0.5 mL). After completion of $NaBH(OAc)_3$ addition, the mixture was stirred for further 12 h. The crude product was purified on a silica gel column (DCM/MeOH = 25:1−8:1). The residue was dissolved in anhydrous DCM (3 mL). HCl (1 N solution in ether, 3 mL) was added dropwise to the above solution and stirred for 30 min at rt. The precipitate was collected and purified by preparative high-performance liquid chromatography to give dihydrochloride dihydrate of **93** (34 mg, 38% yield) as a white solid. mp 100.6−101.7 °C. HPLC purity: 95.78% (method B). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 2H), 7.98 (s, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.80−7.74 (m, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.48−7.42 (m, 2H), 7.38−7.34 (m, 1H), 7.32−7.27 (m, 3H), 7.13 (t, J = 7.6 Hz, 1H), 7.07 (dd, J = 7.4, 1.0 Hz, 1H), 6.87 (d, J = 7.2 Hz, 1H), 6.47 (s, 1H), 6.35 (d, J = 5.6 Hz, 1H), 5.59 (br, 6H), 5.22 (s, 2H), 4.60 (s, 2H), 4.04 (t, J = 4.8 Hz, 2H), 3.66 (t, J = 5.4 Hz, 2H), 3.07 (s, 3H), 2.96−2.85 (m, 2H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.5, 150.8, 142.2, 141.5, 138.8, 136.1, 132.8, 132.7, 132.3, 131.6, 130.9, 129.6, 129.2, 128.2, 126.9, 125.3, 124.9, 118.7, 111.4, 107.6, 104.7, 96.8, 68.3, 56.3, 54.1, 48.3, 44.6, 38.5, 15.7. ESI-HRMS: m/z calculated for $C_{32}H_{33}N_3NaO_2^+$ [M + Na]$^+$, 514.2465; found, 514.2460.

*5-((2-Formyl-5-(hydroxymethyl)phenoxy)methyl)nicotinonitrile (**96**).* 2-Hydroxy-4-(hydroxymethyl)benzaldehyde (compound **95**, 683 mg, 4.49 mmol), K_2CO_3 (683 mg, 4.94 mmol), and 5-(chloromethyl)nicotinonitrile (755 mg, 4.95 mmol) were suspended in MeCN (20 mL) and stirred for 10 h at 60 °C. Then, the reaction mixture was cooled, diluted with H_2O (230 mL), and filtered. The precipitate was washed with water and dried in vacuo to give **96** (1.01 g, 84% yield) as a yellowish solid, which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.41 (d, J = 0.8 Hz, 1H), 9.04 (dd, J = 3.2, 2.0 Hz, 2H), 8.55 (t, J = 2.0, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.29 (d, J = 0.8 Hz, 1H), 7.08 (ddd, J = 8.0, 1.2, 0.8 Hz, 1H), 5.49 (t, J = 5.6 Hz, 1H), 5.39 (s, 2H), 4.59 (d, J = 5.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.1, 160.1, 152.3, 152.2, 151.9, 138.8, 132.9, 128.1, 123.1, 118.8, 116.9, 110.8, 109.1, 66.5, 62.5. ESI-MS: m/z = 269.10 [M + H]$^+$.

*5-((5-(Chloromethyl)-2-formylphenoxy)methyl)nicotinonitrile (**97**).* A solution of MsCl (537 mg, 4.69 mmol) in anhydrous DCM (4 mL) was added dropwise to a solution of **96** (838 mg, 3.12 mmol) and pyridine (494 mg, 6.24 mmol) in anhydrous DCM (20 mL) and anhydrous DMF (2 mL) at 0 °C. After completion of addition, the reaction mixture was stirred for 24 h at rt, and then diluted with water and extracted with DCM. The combined organic phase was washed with water, concentrated in vacuo, and purified on a silica gel column (PE/EA = 2:1) to give **97** (565 mg, 63% yield) as a yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (d, J = 0.4 Hz, 1H), 9.04 (dd, J = 3.6, 2.0 Hz, 2H), 8.56 (t, J = 2.0 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.44 (d, J = 1.6 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 5.41 (s, 2H), 4.83 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.1, 159.9, 152.4, 152.0, 145.9, 139.0, 132.6, 128.6, 124.2, 121.7, 116.8, 114.0, 109.1, 66.7, 45.3. ESI-MS: m/z = 287.05 [M + H]$^+$.

*2-Methyl-[1,1′-biphenyl]-3-amine (**98**).* At an argon atmosphere, 3-bromo-2-methylaniline (2.79 g, 15 mmol), phenylboronic acid (2.74 g, 22.5 mmol), CsOAc (7.20 g, 37.51 mmol), and $PdCl_2$dppf (1.10 g, 1.50 mmol) were suspended in THF (48 mL) and stirred for 24 h at reflux. Then, the mixture was cooled, concentrated in vacuo, diluted with water, and extracted with DCM. The combined organic phase was washed with water and concentrated in vacuo. The crude product was purified by column chromatography to give **98** (2.61 g, 95% yield) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.37 (m, 2H), 7.36−7.27 (m, 3H), 7.07 (t, J = 7.8 Hz, 1H), 6.70 (d, J = 7.6 Hz, 2H), 3.69 (br, 2H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.0, 143.1, 142.4, 129.4, 128.0, 126.7, 126.2, 120.6, 119.9, 114.1, 14.4. ESI-MS: m/z = 184.12 [M + H]$^+$.

*5-((2-Formyl-5-(((2-methyl-[1,1′-biphenyl]-3-yl)amino)methyl)-phenoxy)methyl)nicotinonitrile (**99**).* **97** (397 mg, 1.38 mmol), **98** (379 mg, 2.07 mmol), K_2CO_3 (229 mg, 1.66 mmol), and NaI (42 mg, 0.28 mmol) were suspended in MeCN/DMF (8 mL/2.5 mL) and stirred for 24 h at 30 °C. Then, the reaction mixture was diluted with water and extracted with EA. The combined organic phase was washed with water, concentrated in vacuo, and purified on a silica gel column (PE/EA = 3:2) to give **99** (188 mg, 31% yield) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.46 (s, 1H), 8.87 (d, J = 2.4 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.06 (t, J = 2.2 Hz, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.44−7.39 (m, 2H), 7.37−7.29 (m, 3H), 7.17 (d, J = 8.0 Hz, 1H), 7.09 (t, J = 7.8 Hz, 2H), 6.72 (d, J = 7.6 Hz, 1H), 6.46 (d, J = 8.0 Hz, 1H), 5.24 (s, 2H), 4.50 (s, 2H), 4.19 (br, 1H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.6, 160.2, 152.1, 151.7, 149.3, 145.8, 142.8, 142.3, 137.9, 132.5, 129.9, 129.4, 128.1, 126.8, 126.3, 124.3, 120.5, 120.0, 119.6, 116.3, 111.0, 110.2, 109.2, 66.9, 48.6, 14.4. ESI-MS: m/z = 434.19 [M + H]$^+$.

*(2-((5-Cyanopyridin-3-yl)methoxy)-4-(((2-methyl-[1,1′-biphenyl]-3-yl)amino)methyl)benzyl)-D-serine (**100**).* According to the general procedure C, **99** (278 mg, 0.64 mmol) was reacted with D-serine (135 mg, 1.28 mmol) in anhydrous DMF (6 mL) and anhydrous MeOH (2 mL), which yielded **100** (78 mg, 23% yield) as a white solid. mp 167.1−168.8 °C. HPLC purity: 98.20% (method A). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (d, J = 2.4 Hz, 1H), 8.98 (d, J = 2.0 Hz, 1H), 8.50 (t, J = 2.0 Hz, 1H), 7.43−7.38 (m, 2H), 7.38−7.31 (m, 2H), 7.29−7.24 (m, 2H), 7.20 (s, 1H), 7.03 (d, J = 7.6 Hz, 1H), 6.91 (t, J = 7.8 Hz, 1H), 6.42 (dd, J = 7.6, 0.8 Hz, 1H), 6.37 (d, J = 8.0 Hz, 1H), 5.76 (t, J = 6.0 Hz, 1H), 5.27 (dd, J = 18.8, 12.8 Hz, 2H), 4.37 (d, J = 5.6 Hz, 2H), 4.10 (dd, J = 20.6, 13.4 Hz, 2H), 3.75 (dd, J = 11.2, 4.4 Hz, 1H), 3.63 (dd, J = 11.2, 6.8 Hz, 1H), 3.19 (dd, J = 6.8, 4.4 Hz, 1H), 2.03 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.9, 156.2, 152.4, 151.8, 146.4, 143.3, 142.4, 141.7, 138.9, 133.1, 131.2, 129.2, 128.0, 126.6, 125.8, 120.2, 119.5, 119.0, 117.7, 116.9, 110.9, 108.9, 108.8, 66.3, 62.6, 60.3, 46.6, 45.3, 14.6. ESI-HRMS: m/z calculated for $C_{31}H_{31}N_4O_4^+$ [M + H]$^+$, 523.2340; found, 523.2338.

Biochemical Assay of PD-1/PD-L1 Blockade. The assay was performed using the PD-1/PD-L1 binding assay kit (Cisbio, 64CUS000C-1). BMS202 was purchased from MedChemExpress and used as a positive control. The protocol was performed according to the instructions of the assay kit. Briefly, 2 μL of compounds (or diluent buffer), 4 μL of PD-L1-Tag1, and 4 μL of PD1-Tag2 were incubated in 384-well low volume plates at rt for 15 min. Then, 10 μL of the premixture of two conjugates (5 μL of anti-Tag1-Eu^{3+} and 5 μL of anti-Tag2-XL665) was added to wells. The mixture was incubated at rt for 2.5 h, and the fluorescence emission was detected at two different wavelengths (665 and 620 nm) on an HTRF reader (F200Pro, TECAN). The results were calculated from the 665 and 620 nm fluorescence signals and expressed in an HTRF ratio = (665 nm/620 nm) × 10^4. The inhibitory percentage was calculated from the HTRF ratio. The IC_{50} value was calculated from inhibitory percentage.

Binding Specificity and Affinity Assay. The specificity assay was performed by biolayer interferometry (BLI) using the Octet RED

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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system (ForteBio). The proteins were purchased from the ACROBiosystems Company. Human PD-L1/Fc, PD-1/Fc, PD-L2/Fc, CD47/Fc, TIM-3/Fc, LAG-3/Fc, and CD27/Fc were diluted to 30 μg/mL using running buffer (PBS, 0.02% Tween-20, and 0.1% BSA) and loaded onto anti-human IgG Fc capture (AHC) biosensors for 600 s. Compound **52** was diluted to a concentration of 500 nM (2% DMSO) using running buffer. After loading, the biosensors were baselined, associated in compound **52** for 300 s, and then dissociated in buffer for 300 s. The baseline-corrected binding curves were analyzed.

To determine the binding affinity, the 10 μg/mL biotinylated human PD-L1/AVI (ACRO) was loaded onto SA (streptavidin) biosensors for 300 s (60 s for determination of human PD-1). After loading, the biosensors were baselined, associated in defined concentrations of compounds for 180 s (240 s for determination of human PD-1), and then dissociated in buffer for 300 s (600 s for determination of human PD-1). The assay concentrations of compounds were 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0 nM. The assay concentrations of human PD-1/His_6 were 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 0 nM. Each data set was fitted globally to a 1:1 interaction model to determine the kinetic parameters k_a and k_{dis}. Apparent affinity was then calculated as a ratio (k_a/k_{dis}) of these kinetic constants.

Molecular Docking. The crystal structures of PD-L1 (PDB code 5J89) were obtained from the Protein Data Bank. Molecular docking was performed by the GOLD 5.3 software. For receptors, hydrogen atoms were added, and water molecules and bound ligands were removed. The scoring function was set to ChemPLP. All of the other parameters used in the docking process were set to default values. The best binding modes were chosen to analysis according to docking scores. Figures were created using Pymol version Open-Source 1.6.x.

Flow Cytometry. According to the instructions, the CHO-K1 and CHO-K1-hPD-L1 cells (ATCC) were resuscitated and subcultured, when the cell confluence rate exceeded 80%. The cells in the logarithmic growth phase were digested with trypsin and resuspended in the complete medium. The cells were placed in a tube, centrifuged at 1000 rpm for 5 min, and resuspended in FACS staining buffer (1 × PBS with 2% FBS). 5×10^5 cells/tube were incubated with hPD-1/Fc (final concentration, 10 μg/mL) at 4 °C for 30 min. The dilutions of blocking compounds were added into tubes at a final concentration of 500 nM in 100 μL of FACS staining buffer. The equal volume of buffer was added into tubes in the negative and positive control groups. All tubes were incubated at 4 °C for another 30 min. Then, the cells were washed, resuspended in buffer (95 μL), and incubated with the APC anti-human IgG Fc antibody (5 μL). After incubation, the cells were washed and resuspended in buffer. The fluorescence intensity and the percentage of cells bound with hPD-1 (PCB) were analyzed by flow cytometry. The blocking efficacy (%) = [1 − (the PCB value of compounds groups − the PCB value of negative groups)/(the PCB value of the positive control − the PCB value of negative groups)] × 100%.

Determination of IFN-γ Secretion. Peripheral blood mononuclear cells (PBMCs) were isolated from 10 to 15 mL whole blood of a healthy donor using a human lymphocyte separation fluid (TBD sciences, Cat. LTS1077). The cells were washed twice with normal saline and resuspended in buffer (RPMI 1640 complete medium containing 10% FBS), and the density was measured. The PBMCs (1×10^5 cells in 50 μL buffer) were inoculated in 96-well plates. The specific concentrations of the anti-CD3 antibody and anti-CD28 antibody were mixed in a ratio of 1:1. Antibody mixtures (50 μL) were added to the corresponding wells at the final concentrations of 0.8 μg/mL. At the same time, the gradient PD-L1/FC fusion protein (50 μL/well) was added into the coculture system at a final concentration of 10 μg/mL. Compounds were diluted with buffer and added into the coculture system at final concentrations of 0.1 and 1 μM (50 μL/well). The mixtures were cocultured at 37 °C with a 5% CO_2 cell incubator for 72 h. The supernatant was harvested to determine the expression levels of IFN-γ using the ELISA MAXTM Standard Set Human IFN-γ kit (BioLegend) according to the instructions of the manufacturer.

Cytotoxicity Assay. The cell lines Raji (ATCC), Raw 264.7 (ATCC), and PBMCs (isolated from whole blood of a healthy donor) were resuspended in a complete medium with 10% FBS, and cell density was measured. The cells were plated into 96-well plates at the final density of 1×10^4 cells per well (50 μL) and treated with compounds at different final concentrations (100, 50, 25, 12.5, 6.25, and 3.125 μM) or control buffer (containing 0.2% DMSO) for 24 h at a 37 °C, 5% CO_2 cell incubator. After incubation, 100 μL of CellTiter-Glo Luminescent Solution (Promega) was added to each well and shaken at room temperature for 10 min. Luminescence was measured on a GloMax Navigator System with an integration time of 1 s per well according to the instructions of the manufacturer. The killing rate (%) = $(1 - \mathrm{RLU_{compound}}/\mathrm{RLU_{control}}) \times 100\%$.

In Vivo Pharmacological Study. All animal procedures described in this study were performed in adherence with the guidelines as defined by the Animal Care and Use Committee at the Beijing Institute of Pharmacology & Toxicology. Six-week-old PD-1 humanized female mice (full strain name: C57BL/6J-*Pdcd1*$^{em1(hPDCD1)/Smoc}$) were purchased from the Shanghai Southern Model Animal Center and placed in the SPF animal feeding room for 1 week. At the same time, the PD-L1 genetically engineered colon cancer cell line MC38 (mouse PD-L1 knockout and human PD-L1 knock-in) was subcutaneously inoculated into the mouse near the groin of the hind leg. The amount of inoculated cells was 1×10^6 cells per mouse. The animals are randomly divided into 3 groups (6 mice in each group) according to the size of the tumor using the random number table, when the tumor volume reached 50−150 mm^3. BMS202 (15 mg/kg) and compound **58** (15 mg/kg) were administered at the frequency of once a day for 3 weeks by intraperitoneal administration. The treatment with equal volume of buffer (0.8% DMSO and 7.5% RH40 in normal saline) was used as the vehicle control. Tumor volume and body weight were measured twice every week. The tumor volume was calculated using the equation ($0.5 \times L \times W^2$), where L and W refer to the length and width dimensions of the tumor. At the end of the experiment, the mice were sacrificed with the cervical dislocation method, and the tumors were stripped and weighted. Tumor growth inhibition (TGI) was calculated using the equation: $\mathrm{TGI} = [1 - (T_{final} - T_0)/(C_{final} - C_0)] \times 100\%$, where T_{final} and T_0 were the mean tumor volumes of test groups at the end of the treatment and on the first day of treatment, respectively; C_{final} and C_0 were the mean tumor volumes of the vehicle group at the end of the treatment and on the first day of treatment, respectively.

In Vivo Pharmacokinetic Study in Rats. The pharmacokinetic experiments of **58** in rats were performed by Beijing Pharmaron, Inc. according to the guidelines of the Animal Care and Use Committee of the company. PK was evaluated after a single dose of 4 mg/kg intravenous (iv) and 15 mg/kg oral (po) administration in male Sprague Dawley rats (n = 6, 6−8 weeks age, and 200−300 g weight). Dosing solutions were freshly prepared on the day of administration in 25% MPD/10% RH40/65% water. The time points for blood sample collection were 0.08 (only for iv), 0.25, 0.5, 1, 2, 4, 8, and 24 h post dose. Approximately 0.2 mL of blood was collected at each time point. Each blood sample was transferred into plastic microcentrifuge tubes containing an anticoagulant of EDTA-K2 and mixed well then placed on wet ice prior to centrifugation. Blood samples were centrifuged at 4000g for 5 min at 4 °C to obtain plasma. The samples were stored in a freezer at −75 ± 15 °C prior to analysis. Plasma concentrations of the tested compounds were analyzed by the LC−MS/MS method. The Phoenix WinNonlin 8.0 software was used to calculate all pharmacokinetic parameters.

Statistical Analysis. The IC_{50} of PD-1/PD-L1 blockade and PK parameters were expressed as mean ± SD. The other ones were shown as mean ± SEM. An unpaired t-test was used to determine the statistical significance. *, $p < 0.05$; **, $p < 0.01$.

https://dx.doi.org/10.1021/acs.jmedchem.0c01329
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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01329.

Results of binding specificity assay, NMR spectrum of final compounds, and HPLC spectra of key target compounds (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (no. 81903455), the China Postdoctoral Science Foundation (no. 2017M623412), and the National Science and Technology Major Project (no. 2018ZX09711003-003-001).

■ ABBREVIATIONS

PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; IL-2, interleukin 2; IFN-γ, interferon-γ; mAbs, monoclonal antibodies; HTRF, homogeneous time-resolved fluorescence; BMS, Bristol Myers Squibb; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthalene; BPO, benzoyl peroxide; BLI, biolayer interferometry; AHC, anti-human IgG Fc Capture; hPD-L1, human PD-L1; hPD-1, human PD-1; k_a, association constant; k_{dis}, dissociation constant; CHO-K1-hPD-L1, hPD-L1 overexpressing CHO-K1 cells; PCB, percentage of cells bound with hPD-1; PBMCs, peripheral blood mononuclear cells; MPD, 1,2-propanediol; RH40, cremophor RH40; PE, petroleum ether; EA, ethyl acetate; SA, streptavidin; FBS, fetal bovine serum; FACS, fluorescence activated cell sorting; APC, allophycocyanin; TGI, tumor growth inhibition

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