Journal of Medicinal Chemistry

Article

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Discovery of Novel 2-((Pyridin-3-yloxy)methyl)piperazines as α7 Nicotinic Acetylcholine Receptor Modulators for the Treatment of Inflammatory Disorders

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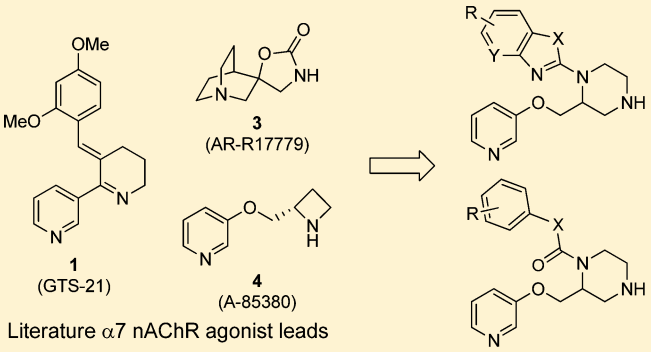
Supporting Information

ABSTRACT: Herein we report the design, synthesis, and structure−activity relationships for a new class of α7 nicotinic acetylcholine receptor (nAChR) modulators based on the 2-((pyridin-3-yloxy)methyl)piperazine scaffold. The oxazolo-[4,5-*b*]pyridine, (***R***)-**18**, and 4-methoxyphenylurea, (***R***)-**47**, were identified as potent and selective modulators of the α7 nAChR with favorable in vitro safety profiles and good oral bioavailability in mouse. Both compounds were shown to significantly inhibit cellular infiltration in a murine model of allergic lung inflammation. Despite the structural and in vivo functional similarities in the compounds, only (***R***)-**18** was shown to be an agonist. Compound (***R***)-**47** demonstrated silent agonist activity. These data support the hypothesis that the anti-inflammatory activity of the α7 nAChR is mediated by a signal transduction pathway that is independent of ion current.



INTRODUCTION

The neuronal nicotinic acetylcholine receptors (nAChRs) are a family of calcium-permeable, ligand-gated ion channels that have received significant attention over the past few years due to their proposed involvement in a variety of disorders. In particular, the α4β2 and α7 subtypes are highly expressed in the brain, and consequently most of this interest has been focused on disorders of the central nervous system (CNS).[1] The α7 nAChR has been implicated in diseases associated with cognitive impairment such as Alzheimer's disease[2] and schizophrenia.[3] Indeed, several α7 agonists, including the partial agonist **1** (GTS-21)[4] and **2** (EVP-6124)[5] (Figure 1), have advanced into the clinic for the treatment of these disorders.

More recent reports have found that the α7 nAChR is expressed on non-neuronal cells,[6] including lymphocytes,[7] macrophages,[8] and intestinal and lung endothelial and epithelial cells.[9] It has been suggested that α7 is an essential regulator of the inflammatory process[10] and that agonists of the receptor may be useful in the treatment of inflammatory conditions such as sepsis,[10a,b,11] postoperative ileus,[12] and ulcerative colitis.[13] Nicotine has been shown to inhibit the lipopolysaccharide (LPS)-induced production of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), in human or murine monocytes.[10a,14] Additionally, both lung expression data and studies with smokers provide a link to the potential utility of α7 nAChR agonists in the treatment of asthma and chronic obstructive pulmonary disease (COPD). While smokers have an increased risk of developing lung cancer, few smokers develop COPD.[15] It has been suggested that the inflammatory response to tobacco smoke is dampened by the anti-inflammatory effects of nicotine.[16] While the original literature data linking the α7 nAChR to inflammation is based on nicotine, a nonselective agonist, more recent work has shown that compound **3** (AR-R17779), a highly selective α7 agonist,[17] is an effective therapy in the collagen induced arthritis model in mice.[18] Furthermore, a group in The Netherlands conducted a phase 1 clinical trial (Clinical Trial Register no. NCT 00783068) with compound **1** in LPS-induced endotoxemia. Although this study showed that at the highest safe dose (150 mg) the reduction in TNF-α, IL-6, IL-1RA, and IL-10 was not statistically significant, there was a correlation between plasma levels of **1** and the levels of TNF-α, IL-6, and IL-1RA.[19]

Furthermore, Targacept has conducted a clinical trial using TC-5987 (structure not disclosed) in asthma. A post-trial press release reported a positive effect on pulmonary function as measured by forced expiratory volume in one second (FEV1), but the full set of results from this study have yet to be published.[20] Here we detail our efforts to develop a new series of selective α7 nAChR modulators to further explore the involvement of this receptor in inflammation.

Received: November 13, 2013
Published: May 9, 2014

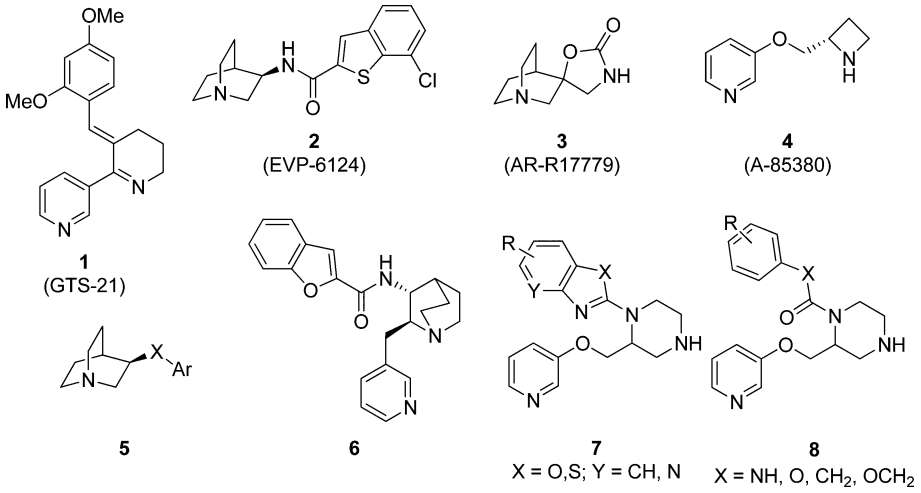
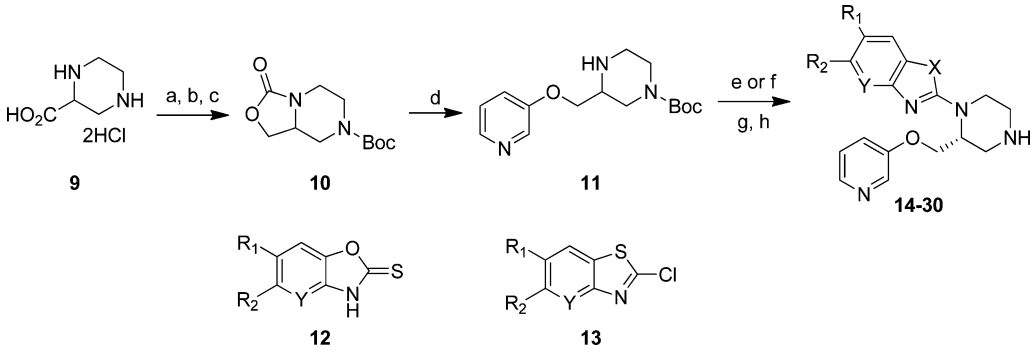


Figure 1. Recent examples of α7 nAChR agonists and design rational.

Scheme 1. Synthetic Route[a]



[a]Reagents and conditions: (a) 2 equiv Boc_2O, Et_3N, MeOH, 50 °C, 96% yield; (b) BH_3−THF, THF, 50 °C; (c) 0.1 equiv NaH, THF, reflux, 80% yield for 2 steps; (d) 10 equiv 3-hydroxypyridine, 10 equiv KOtBu, DMF, 90 °C, 50−80% yield; (e) **12**, toluene, DMSO, 110 °C; (f) **13**, $Pd(OAc)_2$, Ph_3P, NaOtBu, toluene, 110 °C; (g) 4 M HCl in 1,4-dioxane, MeOH; (h) 1 equiv HCl.

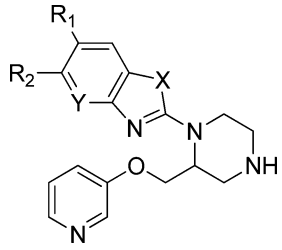
Several reviews have appeared recently outlining the large number of α7 ligands that have been reported over the past decade.[21] Early compounds include the highly selective α7 agonist **3** (AR-R17779),[17] the partial agonist **1**,[22] and the potent $\alpha4\beta2$ agonist **4** (A-85380, K_i = 0.052 nM), which also exhibits a reasonable level of α7 affinity (K_i = 148 nM).[23] Many of the more recent compounds have been based on the quinuclidine scaffold **5**,[24] with variations in the linker and aromatic regions. In comparing these compounds, several structural features are evident, including a basic amine that is protonated at physiological pH, a hydrogen bond acceptor, and a hydrophobic group.[17] Additionally, compounds **1**, **4**, and **6**[24d] have a 3-pyridyl moiety that is also found in nicotine. While the 3-alkoxypyridine portion of compound **4** may serve as the hydrogen bond acceptor and/or the hydrophobic region, the pyridine groups in **1** and **6** appear to occupy an additional region within the ligand binding site. In designing a new class of α7 modulators, we chose to combine all four of these structural features on the piperazine core, leading to the generic compounds **7** and **8** (Figure 1). The N-4 nitrogen of the piperazine would serve as the basic amine, while the N-1 nitrogen allows for convenient introduction of the hydrogen bond acceptor and hydrophobic groups via benzoxazoles and benzothiazoles without the addition of a stereocenter. The 3-pyridyl moiety is readily introduced at the 2-position from piperazine-2-carboxylic acid.

CHEMISTRY

The synthesis of the *N*-1 benzoxazole- and benzothiazole-substituted 2-((pyridin-3-yloxy)methyl)piperazines is outlined in Scheme 1. We have previously reported the synthesis of 2-(phenoxymethyl)piperazines by phenoxide ring-opening of the bicyclic oxazolidinone **10** and utilized this methodology as an efficient method to introduce the 3-alkoxypyridine group while simultaneously unmasking the *N*-1 nitrogen of the piperazine.[25] Compound **10** was readily prepared from piperazine-2-carboxylic acid, **9**, in three steps, and serves as an orthogonally protected precursor without the need for selective protection of the two piperazine nitrogens. Oxazolidinone ring-opening with the in situ generated potassium salt of 3-hydroxypyridine in DMF gave the key piperazine intermediate **11**. The benzoxazole substituents were introduced by treating compound **11** with the benzoxazole-2-thiones **12**. The benzothiazole compounds were prepared via a palladium mediated coupling with **11** and the 2-chlorobenzothiazoles **13**.[26] In both cases, the Boc group was removed with HCl and the compounds were free-based on work-up. While compounds **14**, **15**, and **23** (Table 1) were isolated as the free-base forms, the remaining compounds were converted to the monohydrochloride salts by the addition of one equivalent of HCl. In general, compounds were prepared as racemic mixtures for initial biological evaluation. The individual enantiomers of selected compounds were then prepared as outlined in Scheme

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966−3983

Table 1. In Vitro α7 nAChR Binding Affinity[a,b]



compd no.	X	Y	R_1	R_2	α7[a] IC_{50} (nM) ± SEM
14	O	CH	H	H	106
15	O	CH	OMe	H	22 ± 11
16	O	CH	H	OMe	71 ± 28
17	O	CH	H	Br	75 ± 13
(*R*)-**17**	O	CH	H	Br	67 ± 7
(*S*)-**17**	O	CH	H	Br	3200 ± 370
18	O	N	H	H	4 ± 11
(*R*)-**18**	O	N	H	H	5.5 ± 2.5
(*S*)-**18**	O	N	H	H	1580 ± 50
19	S	CH	H	H	490 ± 160
20	S	CH	OMe	H	79 ± 17
(*R*)-**20**	S	CH	OMe	H	46 ± 14
(*S*)-**20**	S	CH	OMe	H	5100 ± 1100
21	S	CH	H	OMe	107 ± 11
22	S	CH	CH_3	H	85 ± 19
23	S	CH	Cl	H	153 ± 45
24	S	CH	OCF_3	H	270 ± 110
25	S	CH	CF_3	H	338 ± 48
26	S	CH	F	H	132 ± 21
27	S	CF	H	H	17 ± 6
28	S	CF	F	H	19 ± 6
29	S	N	H	H	32 ± 7
30	S	N	Cl	H	4 ± 1

[a]Rat PC-12 cell binding assay, fluorescently labeled α-bungarotoxin, average of three experiments except for **14**, which is the average of two. [b]All compounds were assayed as the mono-HCl salts except for **14**, **19**, and **23**, which were assayed as the free-bases.

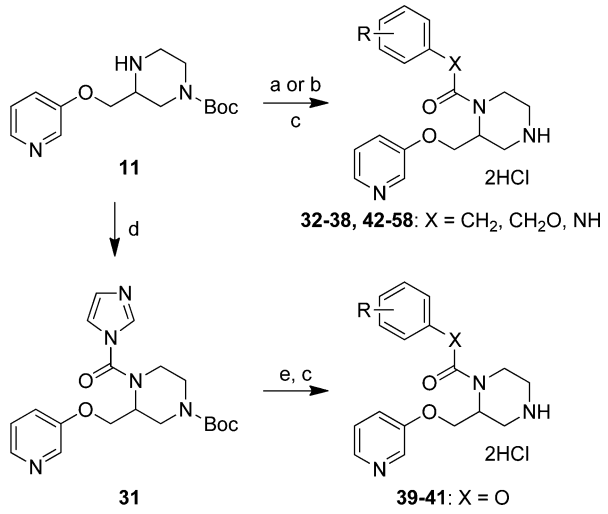
1 starting from the commercially available enantiomers of piperazine-2-carboxylic acid.[27]

Amides, carbamates, and ureas were synthesized as described in Scheme 2. Amide and urea substituents were introduced by reaction of the key piperazine intermediate **11** with either acid chlorides or isocyanates, respectively. The carbamates were prepared by conversion of **11** to the *N*-acylimidazole **31**, followed by displacement with phenols under basic conditions. In general, the Boc group was removed with HCl and the final compounds were isolated as the dihydrochloride salts. Compound **36** was isolated as the free-base upon treatment with aqueous NaOH. As with the benzoxazoles and benzothiazoles, the compounds were first prepared as racemates. Selected active compounds were then constructed as single enantiomers.

RESULTS AND DISCUSSION

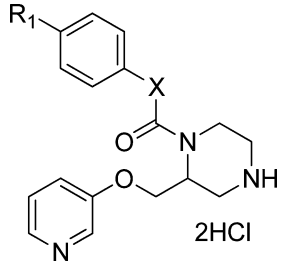
Gratifyingly, our design strategy yielded compounds with very good binding affinity in the α7 nAChR assay as exhibited by the parent benzoxazole and benzothiazole analogues **14** and **19** (Table 1). Moreover, the acylated analogues also showed good binding affinity for the α7 nAChR (Table 2).

Scheme 2. Synthetic Routes[a]



[a]Reagents and conditions: (a) ArCOCl or $ArOCH_2COCl$, CH_2Cl_2; (b) ArNCO, CH_2Cl_2; (c) 4 M HCl in 1,4-dioxane, MeOH; (d) *N*,*N*-carbonyldiimidazole, CH_2Cl_2; (e) ArOH, Et_3N, Cs_2CO_3, CH_3CN, 70 °C.

Table 2. In Vitro α7 nAChR Binding Affinity[a,b]



compd no.	X	R_1	α7[a] IC_{50} (nM) ± SEM
32	bond	H	1080 ± 190
33	bond	Cl	6100 ± 2200
34	CH_2	H	560 ± 70
35	CH_2	Cl	110 ± 10
36	CH_2O	H	2340 ± 110
37	NH	H	680 ± 200
38	NH	Cl	70 ± 40
39	O	H	37 ± 4
40	O	Cl	9.5 ± 0.5
41	O	OMe	11 ± 5

[a]Rat PC-12 cell binding assay, fluorescently labeled α-bungarotoxin, average of at least two experiments. [b]All compounds were assayed as the di-HCl salts except for **36**, which was assayed as the free-base.

In general, the benzoxazole compounds were somewhat more potent than the corresponding benzothiazoles (compounds **14**–**16** vs **19**–**21**). Substitution at the 5- or 6-position led to improved activity in both of the bicyclic ring series, with substitution at the 6-position favored slightly (compounds **15**, **16**, **20**, and **21**). This improvement in binding affinity is also dependent on the electronics of the substituent as exemplified by compounds **20** and **22**–**25**. In these examples, activity decreases as the electron withdrawing ability of the substituent increases (OMe > CH_3 > Cl > OCF_3 > CF_3). There also appears to be a steric component because the strongly electron withdrawing compounds **24** and **26** still exhibit improved binding affinity over the unsubstituted compound **19**. These

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966–3983

results fit nicely into the α7 nAChR pharmacophore model discussed previously wherein the oxazole and thiazole portions of the bicycles act as the hydrogen bond acceptor and the phenyl rings act as the hydrophobic region. Electron donating substituents enhance the hydrogen bonding ability of the benzoxazole and benzothiazole rings, leading to improved potency. The opposite effect on hydrogen bonding ability is true for electron withdrawing groups, leading to reduced affinity, yet this is counterbalanced to some extent by the added hydrophobicity of these substituents. The fact that both heteroatoms in the benzoxazole and benzothiazole rings are capable of hydrogen bonding may also explain why substituents at either the 5- or 6-position are tolerated. The 6-position is slightly favored, probably because the electron donating group is *para* to the nitrogen atom, which has greater hydrogen bonding ability relative to the other heteroatom.

In exploring other substitution on the benzothiazole ring, the 4-fluoro substituted compounds **27** and **28** were found to have significantly improved binding affinities relative to their unsubstituted counterparts, compounds **19** and **26**. On the hypothesis that the aromatic C−F bond might also be involved in a hydrogen bond, the oxazolo[4,5-*b*]pyridine and thiazolo-[4,5-*b*]pyridine derivatives **18**, **29**, and **30** were prepared. Once again, these compounds were found to be more than an order of magnitude more potent than the corresponding benzoxazole and benzothiazoles. As before, the oxygen analogue **18** was more potent than the sulfur compound **29** and a substituent at the 6-position led to improved binding affinity.

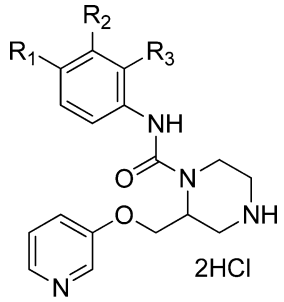
The single enantiomers of compounds **17**, **18**, and **20** were prepared in order to explore the effects of the stereocenter on α7 nAChR activity. The *R*-enantiomers were found to be between 50- and 400-fold more potent than the corresponding *S*-enantiomers. That the *R*-enantiomers are more potent is not surprising, as this enantiomer overlaps better than the *S*-enantiomer with literature compounds **4** and **6** (based on examination of physical models).

To establish that compound (***R***)-**18** is a selective agonist of α7 nAChR, it was evaluated in a voltage clamp electrophysiology study in *Xenopus* oocytes independently expressing either human α7 or human $\alpha4\beta2$ nAChR. These experiments showed that compound (***R***)-**18** is a potent agonist of α7 nAChR with an EC_{50} of 200 nM and an efficacy equivalent to 85% of the maximal acetylcholine response observed at 300 μM.[28] In contrast, no agonist activity on the $\alpha4\beta2$ nAChR was observed.

In the amide series (compounds **32**−**36**), the spacer length between the carbonyl and the phenyl ring had a profound impact on binding affinity, with the phenylacetamides **34** and **35** favored over the benzamides **32** and **33** and the phenoxyacetamide **36**. This is particularly pronounced, with a 4-chloro substituent on the phenyl ring where the phenylacetamide is favored by 60-fold over the corresponding benzamide. The urea analogues **37** and **38** have almost identical binding affinities to the phenylacetamides. In contrast, the carbamate analogues (compounds **39**−**41**) are an order of magnitude more potent than both the phenylacetamides and the ureas. As is the case in the benzoxazole and benzothiazole series, a substituent at the 4-position of the phenyl ring leads to a 4−10-fold improvement in binding affinity. Thus, the 4-substituted phenylcarbamates **40** and **41** have IC_{50}s of 9.5 and 11 nM, respectively, and are in the same potency range as the most potent benzoxazole compound, (***R***)-**18** (IC_{50} = 5.5 nM). Despite their superior potency profiles, we had concerns about possible chemical and metabolic instability of the carbamate group and the possible generation of free phenol in vivo. Ureas are generally more stable than carbamates, and given the sub-100 nM potency of compound **38**, this series was selected for further SAR exploration.

Table 3 provides a summary of the SAR of substitution on the phenyl ring in the urea series. As observed in the benzoxazole and benzothiazole series, substituents at either the 3- or 4-position of the phenyl ring led to improved binding affinity (compounds **38**, **42**, **47**, and **48** vs **37**). This effect was especially pronounced at the 4-position, leading to a 10-fold improvement over the unsubstituted compound. While a substituent at the 2-position was tolerated (compound **49**), no improvement was observed relative to the parent compound. This observation was confirmed with the 2,4-disubstituted compound **50**, which is equipotent to the corresponding 4-substituted compound **47**. Although somewhat small (2−3-fold), there does appear to be a trend toward improved α7 nAChR activity for the 3,4-disubstituted compounds (compounds **43**, **51**, and **54**). With the 3,4-disubstituted bicyclic compound **58**, though, binding affinity was no better than the unsubstituted compound **37**. This was a trend we observed in a number of similar bicyclic compounds (data not shown) and indicates that the constraint locks the

Table 3. In Vitro α7 nAChR Binding Affinity[a,b]



compd no.	R_1	R_2	R_3	$\alpha7^a$ IC_{50} (nM)
37	H	H	H	680 ± 200
38	Cl	H	H	70 ± 40
42	H	Cl	H	210 ± 50
43	Cl	Cl	H	40 ± 10
44	CH_3	H	H	74 ± 11
45	CF_3	H	H	220 ± 20
46	i-Pr	H	H	280 ± 25
47	OMe	H	H	60 ± 20
(***R***)-**47**	OMe	H	H	19 ± 6
(***S***)-**47**	OMe	H	H	18000 ± 5700
48	H	OMe	H	340 ± 10
49	H	H	OMe	850 ± 10
50	OMe	H	OMe	80 ± 30
51	OMe	Cl	H	45 ± 10
52	OCF_3	H	H	152 ± 20
53	Br	H	H	50 ± 20
54	Br	CH_3	H	14 ± 1
55	Br	H	F	42 ± 5
56	Ph	H	H	13 ± 2
57	COPh	H	H	41 ± 24
58	$-OCH_2CH_2-$		H	630 ± 90

[a]Rat PC-12 cell binding assay, fluorescently labeled α-bungarotoxin, average of at least two experiments. [b]All compounds were assayed as the di-HCl salts.

dx.doi.org/10.1021/jm5004599 | *J. Med. Chem.* 2014, 57, 3966−3983

Table 4. In Vitro Selectivity and Safety Profiling[a]

		% inhibition at 10 μM				Cyp % inhibition at 10 μM				
compd no.	α7 IC_{50} (nM)	α4β2	α1β1γδ	5-HT_3	hERG % Inhibition at 1 μM	1A2	2C9	2C19	2D6	3A4
14	106	89	−2	95	22	36	20	24	87	17
(*R*)-**17**	67	96	−3	100	NT	NT	NT	NT	NT	NT
(*R*)-**18**	5.5	58 nM[b]	1	43	72 μM[b]	25	22	9	1	5
(*R*)-**20**	46	83	9	99	36	63	33	78	50	49
27	17	53	20	99	19	63	28	60	60	75
28	19	45	12	91	36	67	26	70	72	83
41	11	90	2	39	NT	NT	NT	NT	NT	NT
47	60	33	−8	23	15	11	14	17	0	7
(*R*)-**47**	19	10	2	7	31 μM[b]	>100 μM all isoforms[b]				
51	45	23	17	40	17	20	10	47	25	66
54	14	15	23	80	27	48	21	51	85	80
55	42	30	7	47	18	13	7	32	41	58
57	41	18	10	16	40	16	62	49	7	23

[a]All assays. except α7, performed at Cerep in duplicate.[31] [b]IC_{50} based on a 5 concentration titration curve. Data reduction performed in SigmaPlot

two substituents into a sterically unfavorable conformation. We also explored the possibility that a hydrogen bond acceptor at the 2-position might lead to a significant improvement in binding affinity as observed in the benzoxazole and benzothiazole series. In the urea series, however, the 2-fluoro compound **55** was equipotent to its unsubstituted counterpart, compound **53**.

In looking at a variety of substituents at the 4-position of the phenyl ring, we found that smaller, electron-donating (compounds **44** and **47**) to mildly electron-withdrawing (compounds **38** and **53**) groups were equipotent in α7 nAChR binding affinities, with IC_{50}s in the 50−75 nM range. Compounds with strongly electron-withdrawing substituents (compounds **45** and **52**), however, were 3−5-fold less potent. Whereas the bulky isopropyl group of compound **46** also resulted in a decrease in binding affinity, the planar phenyl substituent of compound **56** provided the most potent compound in the urea series with an IC_{50} of 13 nM. A number of known α7 modulators also have phenyl groups in this region, indicating that there is a fairly large hydrophobic pocket in the ligand binding site. The benzophenone moiety of compound **57**, with an IC_{50} of 41 nM, also benefits from this added lipophilic interaction and is able to overcome some of the electronic liability of the electron withdrawing carbonyl group. These results again fit nicely into the α7 nAChR pharmacophore discussed earlier wherein the urea carbonyl acts as a hydrogen bond acceptor and the phenyl ring and its substituents occupy a hydrophobic region of the binding pocket.

The single enantiomers of compound **47** were prepared, with the majority of the α7 nAChR activity found in the *R*-enantiomer. Whereas in the benzoxazole and benzothiazole series, the *S*-enantiomers generally bound to α7 in the low micromolar range, compound (*S*)-**47** had an IC_{50} of 18 μM, about 1000-fold less potent than (*R*)-**47** (IC_{50} = 19 nM).

Compound (*R*)-**47** was also evaluated in two voltage clamp experiments using *Xenopus* oocytes independently expressing either α7 or α4β2 nAChRs. As with compound (*R*)-**18**, no agonist activity was observed in cells expressing α4β2 nAChRs. To our surprise, compound (*R*)-**47** did not elicit a current from α7 nAChRs on its own. However, when the oocytes were pretreated with PNU-120596, a type 2 positive allosteric modulator of nAChRs,[29] a current was observed.[28] This type of behavior is consistent with a silent agonist. Thus, compound (*R*)-**47** appears to bypass the open channel state proceeding directly to a desensitized state without the passage of ions.

On the basis of α7 nAChR binding affinities and structural diversity, several compounds were selected for further profiling in selectivity assays against the α4β2 nAChR, the muscle type nAChR, α1β1γδ, and the 5-HT_3 receptor (Table 4). The benzoxazole and oxazolo[4,5-*b*]pyridine compounds **14**, (*R*)-**17**, and (*R*)-**18** all showed nearly complete inhibition of the α4β2 nAChR at a concentration of 10 μM. An IC_{50} of 58 nM was found for (*R*)-**18**, corresponding to a modest 11-fold binding selectivity for the α7 nAChR. This stands in contrast to the functional data showing no agonist activity and indicates that the compound might be an antagonist of the α4β2 nAChR. The benzothiazole compounds (*R*)-**20**, **27**, and **28**, on the other hand, had improved selectivity over the α4β2 nAChR, especially in the latter two cases. In contrast to the benzoxazoles and benzothiazoles, none of the ureas showed significant binding to the α4β2 nAChR at 10 μM. Indeed, compound (*R*)-**47** was found to have an IC_{50} of >100 μM for α4β2, consistent with its lack of functional activity in the voltage clamp experiment. The carbamate analogue, **41**, was less selective for the α4β2 nAChR, exhibiting 90% inhibition at 10 μM. None of the compounds profiled showed any significant inhibition of the muscle-type receptor at 10 μM. For the 5-HT_3 receptor, the ureas and carbamates showed lower inhibition in general than the benzoxazoles and benzothiazoles. Compound (*R*)-**18** was the only urea that exhibited less than 50% inhibition at 10 μM, while compound (*R*)-**47** was the most selective compound, with only 7% inhibition at 10 μM.

In vitro safety was assessed for this set of compounds including human ether-à-go-go-related gene (hERG) and cytochrome P450 (Cyp) inhibition assays. Inhibition of the hERG potassium channel is believed to cause prolongation of the QT interval and to increase the risk of cardiac arrhythmia.[30] Because this class of compounds loosely fits the pharmacophore of known hERG inhibitors, compounds were routinely assessed in a whole-cell voltage-clamp assay at 1 μM. Compounds **14**, (*R*)-**20**, **27**, and **28** showed moderate inhibition, with the benzothiazole compounds having somewhat higher inhibition than the benzoxazole. The oxazolo[4,5-*b*]pyridine (*R*)-**18** was found to have a hERG IC_{50} of 72 μM, indicating a good margin of safety relative to both the α7 nAChR activity (5.5 nM) and the efficacious plasma levels

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966−3983

Table 5. Pharmacokinetic Profile of Compound (*R*)-18 in Mouse after Oral Administration

route	compd	species	dose (mg/kg)	*F* (%)	$T_{1/2}$ (min)	C_{max} (ng/mL)	Cl (mL/min/kg)
IV	(*R*)-**18**	mouse	1		19		72
PO	(*R*)-**18**	mouse	5	50	98	787	
IV	(*R*)-**47**	mouse	1		39		103
PO	(*R*)-**47**	mouse	5	76	104	324	
IV	(*R*)-**47**	rat	1		73		86
PO	(*R*)-**47**	rat	15	89	141	751	

(≤2.5 μM, see below). The urea compounds, **47**, (*R*)-**47**, **51**, **54**, **55**, and **57**, showed modest inhibition of hERG, with the more lipophilic compounds **54**, and **55** inhibiting 27% and 40% of the channel activity, respectively. Compound (*R*)-**47** was further assessed in a five-point assay and was found to have a hERG IC_{50} of 31 μM. This corresponds to a good margin of safety relative to both the α7 nAChR activity (19 nM) and the efficacious plasma levels (≤1 μM, see below).

The compounds were also assayed for inhibition of the five main Cyp isoforms at a concentration of 10 μM. Here, a clear distinction between the benzoxazole and benzothiazole classes was seen. The benzothiazole compounds (*R*)-**20**, **27**, and **28** all showed 50% or greater inhibition of all of the isoforms except 2C9. The benzoxazole **14** only showed significant inhibition of 2D6 (87%), while the oxazolo[4,5-*b*]pyridine (*R*)-**18** was again the cleanest compound with no significant inhibition of any of the five isoforms. In the urea series, compound **54** inhibited 80% of the activity of Cyps 2D6 and 3A4, while compounds **51** and **55** were both fairly clean with only moderate inhibition of Cyp 3A4. Compound **47** separated itself from these two compounds with less than 20% inhibition at all of the five isoforms. (*R*)-**47** was found to have IC_{50}s greater than 100 μM across the profiled Cyps. These differences correlate fairly well with the lipophilicity of the compounds, with improved Cyp profiles observed with decreasing lipophilicity.

On the basis of the initial in vitro ADMET properties, compounds (*R*)-**18** and (*R*)-**47** were assayed in a panel of 55 receptors, ion channels, and transporters (Supporting Information Table S1) as well as 11 enzymes (Supporting Information Table S2). Compound (*R*)-**18** showed some activity in the A_{2a} binding assay (50% inhibition at 10 μM). No other activity was >40%. Compound (*R*)-**47** showed activity in the histamine H_3 receptor (86% at 10 μM) and the sodium channel site 2 (48% at 10 μM). Thus, the off-target activity demonstrated by these compounds was limited and nonoverlapping. Given their favorable activity, selectivity, and in vitro safety profile, compounds (*R*)-**18**, and (*R*)-**47** were selected for in vivo profiling. Compound (*R*)-**18** was found to have acceptable pharmacokinetic (PK) properties upon oral administration in mouse at a 5 mg/kg dose (Table 5). Good oral bioavailability of 50% was observed, with a C_{max} of 787 ng/mL (2.53 μM). Despite the moderate oral half-life of 98 min, plasma concentrations were still well above the α7 nAChR IC_{50} of 5.5 nM after 6 h.

Compound (*R*)-**47** also demonstrated acceptable PK properties upon oral administration in mouse and rat (Table 5) typified by very good oral bioavailability of 76% and 89% in mouse and rat, respectively. The compound is rapidly cleared in both species, leading to fairly short half-lives, although the values are somewhat better upon oral administration. The C_{max} of 324 ng/mL (0.95 μM) in mouse corresponds to an approximately 50-fold excess over the α7 IC_{50}. In a separate experiment, it was found that (*R*)-**47** has no significant brain penetration in mouse, with mean brain/plasma ratios of ≤0.06 at 5, 15, and 30 min postintravenous administration. It has been shown that effects mediated by α7 nAChRs in the periphery may be independent of channel current.[10c,32] For example, NS-6740, a silent agonist of α7,[33] has been shown to inhibit LPS-induced TNF in microglial cells[34] while having no effect on cognition.[35] In addition, after this work was completed, ASM-024, a silent agonist of α7 nAChR (structure not disclosed), has shown efficacy in the murine ovalbumin challenge model of asthma.[36]

Given this data, both compounds (*R*)-**18** and (*R*)-**47** were evaluated in a murine model of allergic lung inflammation.[37] In this model, Balb/c mice were sensitized to ovalbumin on days 1 and 14 via intraperitoneal (IP) injection. On days 29, 30, and 31, the sensitized animals received intranasal ovalbumin challenges. Compound (*R*)-**18** was administered orally 30 min prior to each challenge on days 29−31 at doses of 1 and 5 mg/kg. Eight hours after the third ovalbumin challenge, bronchoalveolar lavage (BAL) fluid was collected and analyzed for cellular infiltration. As seen in Figure 2, the ovalbumin

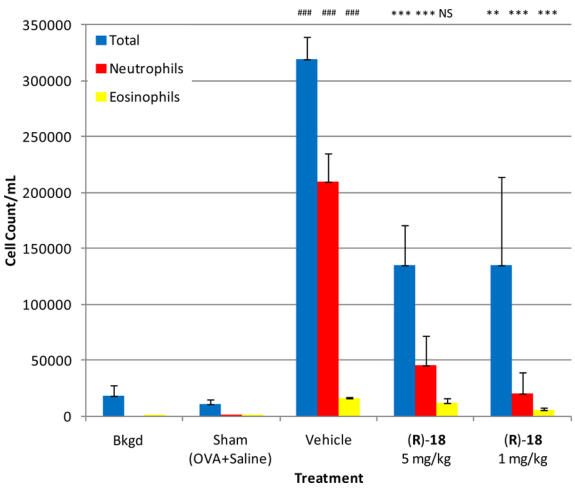


Figure 2. Effect of (*R*)-**18** (QD) in the Balb/c mouse ovalbumin sensitization and challenge model of allergic lung inflammation, with BAL fluid collection 8 h after the final challenge. (###) $p < 0.001$ vs sham control; (**) $p < 0.01$ vs vehicle control; (***) $p < 0.001$ vs vehicle control.

sensitization and challenge leads to a large, mainly neutrophilic cellular infiltration in the BAL fluid at the 8 h time point (vehicle control vs saline/saline control). Although smaller, there is also a statistically significant influx of eosinophils. Compound (*R*)-**18** exhibited statistically significant inhibition of both total cells (60 ± 11%, $p < 0.001$ at 5 mg/kg; 60 ± 23%, $p < 0.01$ at 1 mg/kg) and neutrophils (78 ± 12%, $p < 0.001$ at 5 mg/kg; 90 ± 8%, $p < 0.001$ at 1 mg/kg) at both doses. While the 5 mg/kg dose did not show statistically significant

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966−3983

inhibition of eosinophils, the 1 mg/kg dose inhibited 64 ± 9% ($p < 0.001$) of the eosinophilic infiltration. These data suggest that α7 modulators may have efficacy in various phenotypes of asthma, including those involving infiltration of eosinophils and/or neutrophils.

Compound (*R*)-**47** was similarly evaluated in the Balb/c mouse ovalbumin sensitization and challenge model of allergic lung inflammation in two separate experiments.[37] In the first experiment, compound (*R*)-**47** was administered orally, twice a day, at doses of 0.2, 1, 5, and 30 mg/kg (Figure 3). For the two

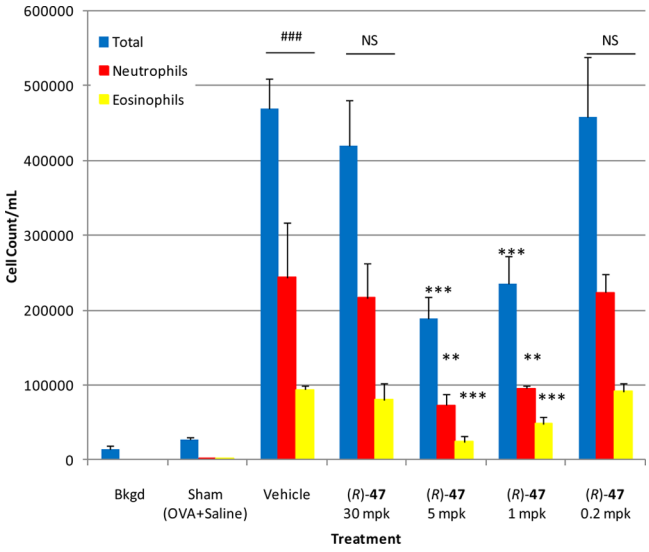


Figure 3. Effect of (*R*)-**47** (BID) in the Balb/c mouse ovalbumin sensitization and challenge model of allergic lung inflammation, with BAL fluid collection 8 h after the final challenge. (###) $p < 0.001$ vs sham control; (**) $p < 0.005$ vs vehicle control; (***) $p < 0.001$ vs vehicle control.

intermediate doses, statistically significant inhibition of total cells (63 ± 6%, $p < 0.001$ at 5 mg/kg; 53 ± 7%, $p < 0.001$ at 1 mg/kg), neutrophils (70 ± 5%, $p < 0.001$ at 5 mg/kg; 61 ± 1%, $p < 0.001$ at 1 mg/kg), and eosinophils (74 ± 8%, $p < 0.001$ at 5 mg/kg; 48 ± 9%, $p < 0.001$ at 1 mg/kg) was observed. The 5 mg/kg dose results are comparable to those observed with compound (*R*)-**18** dosed at 1 mg/kg, while the 1 mg/kg dose of (*R*)-**47** was somewhat less efficacious in inhibiting both neutrophils and eosinophils. This is consistent with the 5-fold difference in potency between the two compounds. No significant inhibition was observed at the lowest dose, likely due to insufficient plasma levels of compound. Similarly, no significant inhibition of cellular infiltration was observed for the 30 mg/kg dose. A possible explanation for this is discussed below.

To further demonstrate that the in vivo efficacy results were mediated through the α7 nAChR, the racemic compound **47** and its two enantiomers, (*R*)-**47** and (*S*)-**47**, were dosed in the ovalbumin sensitization and challenge model (Figure 4). In this experiment, both the racemate dosed at 10 mg/kg and (*R*)-**47** dosed at 1 mg/kg showed statistically significant inhibition of total cells, neutrophils, and eosinophils comparable to the efficacy observed in the previous study. The very weak α7 modulator (*S*)-**47**, on the other hand, showed no significant inhibition of neutrophils or eosinophils at 1 mg/kg relative to vehicle. This finding further supports the theory that the anti-

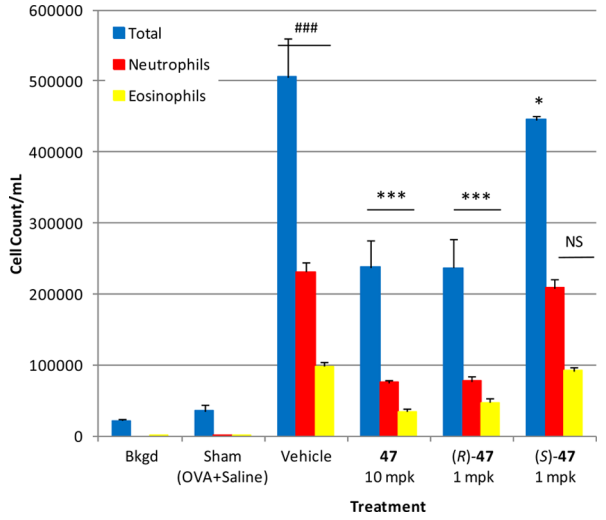


Figure 4. Effect of **47**, (*R*)-**47**, and (*S*)-**47** (BID) in the Balb/c mouse ovalbumin sensitization and challenge model of allergic lung inflammation, with BAL fluid collection 8 h after the final challenge. (###) $p < 0.001$ vs sham control; (*) $p < 0.05$ vs vehicle control; (**) $p < 0.005$ vs vehicle control; (***) $p < 0.001$ vs vehicle control.

inflammatory effects of (*R*)-**47** are mediated through the α7 nAChR.

The question remains as to why (*R*)-**47** showed reduced efficacy when dosed at 30 mg/kg in the ovalbumin model. Papke and co-workers[38] have shown that α7 nAChRs have at least two distinct desensitized states, D_s and D_i. Furthermore, high agonist concentrations appear to stabilize the D_i state. We can thus hypothesize that (*R*)-**47** at low concentrations induces a desensitized state that is competent to initiate the anti-inflammatory effect. However, at high concentrations, the compound stabilizes a distinct desensitized state that is incompetent with respect to anti-inflammatory activity. Although we cannot rigorously exclude an unknown mechanism of action, the close structural similarity, selectivity profile, and in vivo efficacy of these compounds supports the hypothesis that α7 nAChRs are important mediators of the anti-inflammatory response in the murine ovalbumin challenge model of asthma, in an ion current independent manner.

CONCLUSION

The design, synthesis, SAR, and in vitro and in vivo profiles of N-1 substituted 2-((pyridin-3-yloxy)methyl)piperazines as α7 nAChR modulators have been described. Of these compounds, the oxazolo[4,5-*b*]pyridine (*R*)-**18** and methoxyphenyl urea (*R*)-**47** were found to be potent modulators of the α7 nAChR with good selectivity over other nAChRs. These compounds also exhibited a favorable in vitro safety profile and good oral bioavailability. Additionally, both compounds were shown to significantly decrease cellular infiltration in the Balb/c mouse ovalbumin sensitization and challenge model of allergic lung inflammation at oral doses down to 1 mg/kg with no associated adverse effects. Despite the similarity in these compounds, only (*R*)-**18** is a true agonist, while (*R*)-**47** is a silent agonist. These results provide added support to the theory that the α7 nAChR is an essential regulator of the inflammatory process and that this effect is likely independent of ion current. Nonetheless, a selective agonist of the receptor may be useful in the treatment of inflammatory conditions.

dx.doi.org/10.1021/jm5004599 | *J. Med. Chem.* 2014, 57, 3966−3983

EXPERIMENTAL SECTION

General. Air and moisture sensitive liquids and reagents were transferred via syringe or cannula and were introduced into oven-dried glassware under a positive pressure of dry nitrogen through rubber septa. All reactions were stirred magnetically. Commercial reagents were used without further purification. Analytical thin-layer chromatography was performed on EM Science precoated glass-backed silica gel 60 Å F-254 250 μm plates. Visualization of the plates was effected by one or more of the following techniques: (a) ultraviolet illumination, (b) exposure to iodine vapor, and/or (c) immersion of the plate in a 10% solution of phosphomolybdic acid (PMA) in ethanol followed by heating. Column chromatography was performed on a FlashMaster Personal or FlashMaster Personal Plus system using ISOLUTE Flash Si II silica gel prepacked cartridges (available from Biotage). Preparative reversed-phase HPLC chromatography (HPLC) was accomplished using an Agilent 1100 series system and an Agilent Prep-C18 (21.2 mm I.D. × 150 mm) column equipped with an Agilent Prep-C18 (21.2 mm I.D.) guard column. The mobile phase used was a mixture of H_2O (A) and MeCN (B) containing 0.1% TFA. ^{1}H NMR spectra were recorded on a Varian Gemini 2400 (400 MHz) spectrometer and are reported in ppm using residual solvent as the internal standard ($CDCl_3$ at 7.24 ppm or DMSO-d_6 at 2.50 ppm). ^{13}C NMR spectra were recorded on a Varian Gemini 2400 (100 MHz) spectrometer and are reported in ppm using residual solvent as the internal standard ($CDCl_3$ at 77.2 ppm or DMSO-d_6 at 39.5 ppm). High performance liquid chromatography–electrospray mass spectra (LC-MS) were obtained using an Agilent 1100 series HPLC equipped with a binary pump, a diode array detector monitored at 254 and 214 nm, an Agilent Eclipse XDB-C8 (4.6 mm I.D. × 150 mm, 5 μm) column, and an Agilent 1100 series LC/MSD mass spectrometer with electrospray ionization. Spectra were scanned from 100 to 1000 amu. The eluent was a mixture of H_2O (A) and MeCN (B) containing 0.1% AcOH at a flow rate of 1 mL/min. A typical gradient was: (a) time = 0, 90% A, 10% B; (b) time = 9 min, 10% A, 90% B; (c) time = 9.5 min, 90% A, 10% B; (d) time = 12 min, 90% A, 10% B. Purity of the final compounds was assessed by two orthogonal HPLC methods using the Agilent 1100 series HPLC described above. Method A: Agilent Eclipse XDB-C8 (4.6 mm I.D. × 150 mm, 5 μm) column, gradient elution of H_2O with 0.1% AcOH (A) and MeOH (B) at 1.5 mL/min, (a) time = 0, 95% A, 5% B; (b) time = 11 min, 100% B; (c) time =12 min, 95% A, 5% B; (d) time = 14.5 min, 95% A, 5% B. Method B: Agilent Zorbax Eclipse XDB-C18 (4.6 mm I.D. × 150 mm, 5 μm) column, gradient elution of H_2O with 0.1% TFA (A) and MeCN with 0.1% TFA (B) at 1.5 mL/min, (a) time = 0, 95% A, 5% B; (b) time = 11 min, 100% B; (c) time = 12 min, 95% A, 5% B; (d) time = 14.5 min, 95% A, 5% B. All compounds submitted for biological assays showed ≥95% purity. Elemental analysis (C, H, N) for select compounds was performed by Atlantic Microlab, Inc. (Norcross, Georgia).

1,4-Bis(tert-butoxycarbonyl)piperazine-2-carboxylic Acid. According to the literature procedure,[39] a solution of di-*tert*-butyldicarbonate (63 g, 290 mmol) in MeOH (100 mL) was added portionwise to a solution of piperazine-2-carboxyilc acid dihydrochloride (25.0 g, 123 mmol) and triethylamine (48 mL, 340 mmol) in MeOH (150 mL) over 30 min. Upon complete addition, the reaction mixture was heated to 50 °C for 2 h. Upon cooling to rt, the reaction mixture was concentrated under reduced pressure. The material was dissolved in water (300 mL), and the solution was brought to pH 2 with 1 M aqueous HCl. This was extracted with EtOAc (4 × 200 mL), and the combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure until ∼100 mL EtOAc remained. The solution was diluted with hexanes (150 mL) and cooled to 0 °C. The resulting solid was collected by filtration, washed with hexanes (2×), and air-dried. This gave 38.9 g (96%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02–12.80 (br, 1H), 4.50–4.24 (m, 2H), 3.94–3.72 (br, 1H), 3.66 (d, J = 12.8 Hz, 1H), 3.22–2.92 (m, 2H), 2.90–2.68 (br, 1H), 1.42–1.34, (m, 18H).

*tert-Butyl 3-Oxotetrahydro-1H-oxazolo[3,4-a]pyrazine-7(3H)-carboxylate (**10**).* Borane–THF complex (1.0 M solution in THF, 200 mL, 200 mmol) was added slowly to a solution of 1,4-bis(*tert*-butoxycarbonyl)piperazine-2-carboxylic acid (30.3 g, 91.7 mmol) in THF (100 mL). Upon complete addition, the reaction mixture was heated to 50 °C for 2 h. Upon cooling to rt, the reaction mixture was carefully quenched by the dropwise addition of MeOH. After gas evolution ceased, the reaction mixture was concentrated under reduced pressure. The material was dissolved in EtOAc (300 mL) and washed with 1 N NaOH (2 × 200 mL) and brine (200 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was twice dissolved in THF (50 mL) and concentrated under reduced pressure. The material was dissolved in THF (200 mL), and NaH (60% dispersion in mineral oil, 0.366 g, 0.916 mmol) was added portionwise. The reaction mixture was heated to reflux. After 1 h, the reaction mixture was allowed to cool to rt and was concentrated under reduced pressure. The material was dissolved in EtOAc (300 mL), washed with water (2 × 200 mL) and brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting solid was dissolved in EtOAc (200 mL) with heating, diluted with hexanes (200 mL), and allowed to cool to rt. The white, crystalline solid was collected by filtration after 5 h, washed with hexanes (2×), and dried in the fritted funnel under vacuum. This gave 13.29 g (60%) of the product. The filtrate was concentrated under reduced pressure, dissolved in EtOAc (50 mL) with heating, and diluted with hexanes (200 mL). This was allowed to cool to rt and sit over the weekend. The white, crystalline solid was collected by filtration, washed with hexanes (2×), and dried in the fritted funnel under vacuum. This gave an additional 4.49 g (20%) of the product. R_f = 0.43 in 80% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.41 (t, J = 8.4 Hz, 1H), 4.35–3.98 (br, 2H), 3.92 (dd, J = 5.6 and 8.8 Hz, 1H), 3.80–3.72 (m, 2H), 2.98 (dt, J = 3.6 and 12.4 Hz, 1H), 2.86–2.70 (br, 1H), 2.70–2.55, (br, 1H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 154.2, 81.1, 65.5, 52.9, 47.7 (br), 43.4 (br), 41.1, 28.7. LC-MS: RT = 6.46 min; $[M + Na]^+$ = 264.9. Anal. Calcd for $C_{11}H_{18}N_2O_4$: C, 54.53; H, 7.49; N, 11.56. Found: C, 54.38; H, 7.44; N, 11.35.

*tert-Butyl 3-((Pyridin-3-yloxy)methyl)piperazine-1-carboxylate (**11**).* Potassium *tert*-butoxide (1.18 g, 10.5 mmol) was added to a solution of 3-hydroxypyridine (0.979 g, 10.3 mmol) in DMF (10 mL). After 30 min, compound **10** (0.500 g, 2.06 mmol) was added, and the reaction mixture was heated to 90 °C. After 48 h, a second batch of 3-hydroxypyridine potassium salt was prepared by adding potassium *tert*-butoxide (1.18 g, 10.5 mmol) to a solution of 3-hydroxypyridine (0.979 g, 10.3 mmol) in DMF (5 mL). This was added to the reaction mixture, and heating was continued at 90 °C for 3 days. Upon cooling to rt, water (50 mL) was added, and the reaction mixture was extracted with EtOAc (3 × 500 mL). The combined extracts were washed with 1 N NaOH (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chomatography (0–4% MeOH in CH_2Cl_2 with 0.5% NH_4OH gradient) gave 0.487 g (80%) of the desired product as a white solid. R_f = 0.32 in 10% MeOH in CH_2Cl_2 (faint UV, visualized with PMA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (dd, J = 2.4 and 0.8 Hz, 1H), 8.17 (dd, J = 4.2 and 1.4 Hz, 1H), 7.18–7.10 (m, 2H), 3.96–3.80 (m, 4H), 3.09–3.3.0 (m, 1H), 2.96 (br d, J = 11.6 Hz, 1H), 2.87 (br t, J = 12.0 Hz, 1H), 2.80–2.56 (m, 2H), 2.40–2.20 (br, 1H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1, 154.7, 142.5, 138.0, 123.8, 121.0, 80.0, 69.9, 54.0, 46.0 (br), 45.1, 44.4 (br), 28.7. LC-MS: RT = 3.81 min; $[M + H]^+$ = 294.1.

*2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]oxazole (**14**).* Palladium(II) acetate (18 mg, 0.082 mmol) was added to a mixture of compound **11** (478 mg, 1.63 mmol), 2-chlorobenzoxazole (378 mg, 2.45 mmol), sodium *tert*-butoxide (172 mg, 1.79 mmol), and triphenylphosphine (21 mg, 0.082 mmol) in toluene (5 mL). The reaction mixture was heated to 110 °C overnight. Upon cooling to rt, the reaction mixture was diluted with EtOAc (50 mL) and washed with water (30 mL) and brine (30 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (0–4% MeOH in CH_2Cl_2 gradient), yielding 121 mg (18%) of a thick oil. R_f = 0.40 in 10% MeOH in CH_2Cl_2. LC-MS: RT = 8.43 min, $[M + H]^+$ = 411.2.

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966–3983

Then 4 M HCl in 1,4-dioxane (6 mL, 24 mmol) was added to a solution of the intermediate in MeOH (1 mL). After 2 h, the reaction mixture was concentrated under reduced pressure. The material was dissolved in EtOAc, washed with 1 N NaOH (25 mL) and brine (25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This gave 69.7 mg (76%) of the title compound as a sticky solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22−8.18 (br, 1H), 8.17−8.13 (br, 1H), 7.42−7.27 (m, 4H), 7.15 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.8 Hz, 1H), 4.53−4.45 (m, 2H), 4.42−4.34 (m, 1H), 3.97−3.90 (m, 1H), 3.35−3.25 (m, 1H), 3.22−3.15 (m, 1H), 3.06−2.99 (m, 1H), 2.95 (dd, J = 12.8 and 2.8 Hz, 1H), 2.76 (dt, J = 12.8 and 3.2 Hz, 1H). LC-MS: RT = 3.98 min, $[M + H]^+$ = 311.1. Purity >95% by HPLC method A, RT = 3.63 min. Purity >95% by HPLC method B, RT = 4.71 min.

*6-Methoxy-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]oxazole Hydrochloride (**15**).* A solution of 6-methoxybenzo[d]oxazole-2(3H)-thione[40] (222 mg, 1.23 mmol) and compound **11** (300 mg, 1.02 mmol) in toluene (3 mL) and DMSO (0.3 mL) was heated to 110 °C overnight. Upon cooling to rt, the reaction mixture was concentrated under reduced pressure. The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 151 mg (22%, di-TFA salt) of a thick oil. LC-MS: RT = 8.29 min, $[M + H]^+$ = 441.2. Then 4 M HCl in 1,4-dioxane (6 mL, 24 mmol) was added to a solution of the intermediate in MeOH (1 mL). After 1 h, the reaction mixture was concentrated under reduced pressure and purified by HPLC (5−50% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). The fractions containing the desired product were combined, brought to pH 12 with 1 N NaOH, and were extracted with EtOAc (3×). The combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was dissolved in MeOH (1 mL), and 4 M HCl in 1,4-dioxane (1 equiv HCl) was added. The mixture was concentrated under reduced pressure to give 37.4 mg (44%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60−9.46 (br, 1H), 9.20−9.06 (br, 1H), 8.28 (d, J = 3.2 Hz, 1H), 8.22 (dd, J = 4.6 and 1.4 Hz, 1H), 7.48−7.36 (m, 2H), 7.24 (d, J = 8.8 Hz, 1H), 7.10 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.8 and 2.4 Hz, 1H), 4.80−4.72 (m, 1H), 4.58−4.49 (m, 2H), 4.20−4.13 (m, 1H), 3.75 (s, 3H), 3.60−3.34 (m, 4H), 3.24−3.12 (m, 1H). LC-MS: RT = 4.00 min, $[M + H]^+$ = 341.1. Purity >95% by HPLC method A, RT = 3.73 min. Purity >95% by HPLC method B, RT = 4.88 min.

*5-Methoxy-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]oxazole Hydrochloride (**16**).* The compound was prepared as described for **15** from 5-methoxybenzo[d]oxazole-2(3H)-thione[40] (370 mg, 2.05 mmol) and compound **11** (200 mg, 0.682 mmol). The *N*-Boc intermediate was purified by column chromatography (30−50% EtOAc in hexanes gradient), yielding 243 mg (81%) of a thick oil. R_f = 0.31 in 70% EtOAc in hexanes. LC-MS: RT = 8.37 min, $[M + H]^+$ = 441.2. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 120 mg (58%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70−9.55 (br, 1H), 9.42−9.26 (br, 1H), 8.15 (d, J = 2.8 Hz, 1H), 8.07 (dd, J = 4.6 and 1.0 Hz, 1H), 7.38−7.33 (m, 1H), 7.27 (dd, J = 8.6 and 4.6 Hz, 1H), 7.13 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 2.8 Hz, 1H), 6.46 (dd, J = 8.8 and 2.8 Hz, 1H), 4.67−4.59 (m, 1H), 4.50−4.38 (m, 2H), 4.08−4.00 (m, 1H), 3.58 (s, 3H), 3.48−3.34 (m, 2H), 3.26−3.17 (m, 2H), 3.06−2.93 (m, 1H). LC-MS: RT = 4.09 min, $[M + H]^+$ = 341.1. Purity >95% by HPLC method A, RT = 3.69 min. Purity >95% by HPLC method B, RT = 4.88 min.

*5-Bromo-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]oxazole Hydrochloride (**17**).* The compound was prepared as described for **15** from 5-bromobenzo[d]oxazole-2(3H)-thione[40] (784 mg, 3.41 mmol) and compound **11** (200 mg, 0.682 mmol). The *N*-Boc intermediate was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 310 mg (63%, di-TFA salt) of an off-white solid. LC-MS: RT = 9.49 min, $[M + H]^+$ = 489.0. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 103 mg (56%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80−9.66 (br, 1H), 9.48−9.34 (br, 1H), 8.32 (d, J = 2.4 Hz, 1H), 8.25 (dd, J = 4.8 and 1.2 Hz, 1H), 7.56−7.50 (m, 2H), 7.45 (dd, J = 8.6 and 4.6 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.22 (dd, J = 8.4 and 2.4 Hz, 1H), 4.86−4.79 (m, 1H), 4.68−4.54 (m, 2H), 4.26−4.18 (m, 1H), 3.67−3.52 (m, 2H), 3.46−3.34 (m, 2H), 3.24−3.12 (m, 1H). LC-MS: RT = 4.74 min, $[M + H]^+$ = 388.9. Purity >95% by HPLC method A, RT = 4.47 min. Purity >95% by HPLC method B, RT = 6.00 min.

*(R)-5-Bromo-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]oxazole Hydrochloride ((**R**)-**17**).* The compound was prepared as described for compound **17** from (***R***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68−9.44 (br, 2H), 8.22 (d, J = 2.4 Hz, 1H), 8.18 (dd, J = 4.8 and 1.2 Hz, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.42−7.36 (m, 2H), 7.33 (dd, J = 4.4 and 3.6 Hz, 1H), 7.22 (dd, J = 8.6 and 2.2 Hz, 1H), 4.85−4.78 (m, 1H), 4.67−4.50 (m, 2H), 4.26−4.18 (m, 1H), 3.66−3.51 (m, 2H), 3.43−3.34 (m, 2H), 3.16 (dt, J = 12.4 and 3.6 Hz, 1H). LC-MS: RT = 4.71 min, $[M + H]^+$ = 388.9. Purity >95% by HPLC method A, RT = 4.41 min. Purity >95% by HPLC method B, RT = 6.72 min.

*(S)-5-Bromo-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]oxazole Hydrochloride ((**S**)-**17**).* The compound was prepared as described for compound **17** from (***S***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80−9.66 (br, 1H), 9.48−9.28 (br, 1H), 8.25 (d, J = 2.4 Hz, 1H), 8.20 (dd, J = 4.8 and 1.2 Hz, 1H), 7.54 (d, J = 1.6 Hz, 1H), 7.44−7.39 (m, 2H), 7.36 (dd, J = 4.4 and 3.6 Hz, 1H), 7.22 (dd, J = 8.6 and 2.2 Hz, 1H), 4.86−4.78 (m, 1H), 4.66−4.50 (m, 2H), 4.26−4.18 (m, 1H), 3.66−3.52 (m, 2H), 3.46−3.34 (m, 2H), 3.23−3.10 (m, 1H). LC-MS: RT = 4.67 min, $[M + H]^+$ = 388.9. Purity >95% by HPLC method A, RT = 4.25 min. Purity >95% by HPLC method B, RT = 6.53 min.

*2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)oxazolo[4,5-b]pyridine Hydrochloride (**18**).* The compound was prepared as described for **15** from oxazolo[4,5-b]pyridine-2(3H)-thione[40] (369 mg, 2.42 mmol) and compound **11** (237 mg, 0.808 mmol). The *N*-Boc intermediate was purified by column chromatography (30−100% EtOAc in hexanes gradient), yielding 273 mg (82%) of an off-white solid. R_f = 0.30 in 10% MeOH in EtOAc. LC-MS: RT = 6.59 min, $[M + H]^+$ = 412.7. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 148 mg (64%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10−9.55 (br, 2H), 8.29 (d, J = 2.8 Hz, 1H), 8.21 (dd, J = 4.6 and 1.2 Hz, 1H), 8.18 (dd, J = 5.2 and 1.2 Hz, 1H), 7.78 (dd, J = 7.8 and 1.6 Hz, 1H), 7.48 (ddd, J = 8.4, 2.8, and 1.6 Hz, 1H), 7.39 (dd, J = 8.6 and 4.6 Hz, 1H), 7.07 (dd, J = 7.8 and 5.2 Hz, 1H), 4.92−4.85 (m, 1H), 4.72 (dd, J = 9.8 and 7.4 Hz, 1H), 4.60 (dd, J = 10.6 and 7.0 Hz, 1H), 4.29 (dd, J = 14.8 and 3.2 Hz, 1H), 3.72−3.62 (m, 1H), 3.60−3.54 (m, 1H), 3.46−3.36 (m, 2H), 3.24−3.13 (m, 1H). LC-MS: RT = 2.18 min, $[M + H]^+$ = 312.5. Purity >95% by HPLC method A, RT = 1.62 min. Purity >95% by HPLC method B, RT = 4.10 min.

*(R)-2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)oxazolo[4,5-b]pyridine Hydrochloride ((**R**)-**18**).* The compound was prepared as described for compound **18** from (***R***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92−9.74 (br, 1H), 9.60−9.44 (br, 1H), 8.30 (d, J = 2.8 Hz, 1H), 8.22 (dd, J = 4.6 and 1.2 Hz, 1H), 8.19 (dd, J = 5.2 and 1.2 Hz, 1H), 7.78 (dd, J = 7.8 and 1.6 Hz, 1H), 7.52−7.46 (m, 1H), 7.40 (dd, J = 8.6 and 4.6 Hz, 1H), 7.07 (dd, J = 7.8 and 5.2 Hz, 1H), 4.92−4.85 (m, 1H), 4.70 (dd, J = 10.4 and 7.2 Hz, 1H), 4.58 (dd, J = 10.4 and 7.2 Hz, 1H), 4.29 (dd, J = 14.8 and 3.2 Hz, 1H), 3.72−3.62 (m, 1H), 3.60−3.54 (m, 1H), 3.46−3.35 (m, 2H), 3.24−3.13 (m, 1H). LC-MS: RT = 3.15 min, $[M + H]^+$ = 312.1. Purity >95% by HPLC method A, RT = 2.67 min. Purity >95% by HPLC method B, RT = 4.14 min.

*(S)-2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)oxazolo[4,5-b]pyridine Hydrochloride ((**S**)-**18**).* The compound was prepared as described for compound **18** from (***S***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00−9.82 (br, 1H), 9.70−9.50 (br, 1H), 8.36 (d, J = 2.8 Hz, 1H), 8.26 (dd, J = 4.6 and 1.2 Hz, 1H), 8.19 (dd, J = 5.2 and 1.2 Hz, 1H), 7.79 (dd, J = 7.8 and 1.6 Hz, 1H), 7.62−7.56 (m, 1H), 7.48 (dd, J = 8.6 and 4.6 Hz, 1H), 7.08 (dd, J = 7.8 and 5.2 Hz, 1H), 4.94−4.86 (m, 1H), 4.72 (dd, J = 10.4 and 7.2 Hz, 1H), 4.62 (dd, J = 10.4 and 7.2 Hz, 1H), 4.30 (dd, J = 14.8 and 3.2 Hz, 1H), 3.74−3.62 (m, 1H), 3.62−3.54 (m, 1H), 3.46−3.36 (m, 2H), 3.26−3.14 (m, 1H).

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966−3983

LC-MS: RT = 2.92 min, $[M + H]^+$ = 312.1. Purity >95% by HPLC method A, RT = 2.50 min. Purity >95% by HPLC method B, RT = 4.04 min.

*2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]thiazole (**19**).* The compound was prepared as described for compound **14** using 2-chlorobenzothiazole (87 mg, 0.51 mmol) and compound **11** (100 mg, 0.341 mmol). The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 116 mg (52%, di-TFA salt) of a thick oil. LC-MS: RT = 9.14 min, $[M + H]^+$ = 427.1. The material was deprotected as described for **14** to gave 41.7 mg (59%) of the title compound as a sticky solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, J = 3.2 Hz, 1H), 8.17 (d, J = 4.4 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.52−7.42 (m, 2H), 7.34 (dd, J = 8.6 and 4.6 Hz, 1H), 7.27 (t, J = 7.4 Hz, 1H), 7.06 (t, J = 7.4 Hz, 1H), 4.54 (t, J = 9.0 Hz, 1H), 4.42−4.34 (m, 1H), 4.26 (dd, J = 9.6 and 5.6 Hz, 1H), 3.76−3.66 (m, 1H), 3.42−3.28 (m, 1H), 3.15 (d, J = 12.4 Hz, 1H), 3.02−2.95 (m, 1H), 2.85 (dd, J = 12.6 and 3.4 Hz, 1H), 2.71 (dt, J = 12.4 and 3.6 Hz, 1H). LC-MS: RT = 4.38 min, $[M + H]^+$ = 327.1. Purity >95% by HPLC method A, RT = 3.98 min. Purity >95% by HPLC method B, RT = 5.23 min.

*6-Methoxy-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo-[d]thiazole Hydrochloride (**20**).* The compound was prepared as for compound **14** using 2-chloro-6-methoxybenzo[d]thiazole (1.02 g, 5.12 mmol) and compound **11** (1.00 g, 3.41 mmol). The material was purified by column chromatography (20−60% EtOAc in hexanes gradient), yielding 1.145 g (73%) of a tan solid. R_f = 0.29 in 70% EtOAc in hexanes. LC-MS: RT = 8.90 min, $[M + H]^+$ = 457.0. The material was deprotected and converted to the HCl salt as described for compound **15**, yielding 828.6 mg (88%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78−9.62 (br, 1H), 9.46−9.30 (br, 1H), 8.39 (d, J = 2.8 Hz, 1H), 8.24 (d, J = 4.8 Hz, 1H), 7.62−7.55 (m, 1H), 7.50−7.38 (m, 3H), 6.91 (dd, J = 8.8 and 2.4 Hz, 1H), 4.76−4.69 (m, 1H), 4.62 (t, J = 9 Hz, 1H), 4.52 (dd, J = 10.0 and 6.0 Hz, 1H), 4.08−4.00 (m, 1H), 3.76 (s, 3H), 3.67−3.34 (m, 4H), 3.24−3.10 (m, 1H). LC-MS: RT = 4.37 min, $[M + H]^+$ = 357.0. Purity >95% by HPLC method A, RT = 4.02 min. Purity >95% by HPLC method B, RT = 5.28 min.

*(R)-6-Methoxy-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)-benzo[d]thiazole Hydrochloride ((**R**)-**20**).* The compound was prepared as described for compound **20** from (***R***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82−9.70 (br, 1H), 9.54−9.38 (br, 1H), 8.39 (br, 1H), 8.24 (d, J = 3.6 Hz, 1H), 7.60−7.55 (m, 1H), 7.50−7.38 (m, 3H), 6.91 (dd, J = 8.8 and 2.4 Hz, 1H), 4.76−4.68 (m, 1H), 4.62 (t, J = 9 Hz, 1H), 4.56−4.48 (m, 1H), 4.08−3.98 (m, 1H), 3.76 (s, 3H), 3.68−3.52 (m, 2H), 3.42−3.32 (m, 2H), 3.22−3.10 (m, 1H). LC-MS: RT = 4.80 min, $[M + H]^+$ = 357.0. Purity >95% by HPLC method A, RT = 4.03 min. Purity >95% by HPLC method B, RT = 6.05 min.

*(S)-6-Methoxy-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)-benzo[d]thiazole Hydrochloride ((**S**)-**20**).* The compound was prepared as described for compound **20** from (***S***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78−9.65 (br, 1H), 9.45−9.29 (br, 1H), 8.36 (br, 1H), 8.24−8.19 (m, 1H), 7.56−7.50 (m, 1H), 7.48−7.36 (m, 3H), 6.94−6.88 (m, 1H), 4.76−4.68 (m, 1H), 4.60 (t, J = 9 Hz, 1H), 4.54−4.46 (m, 1H), 4.08−3.99 (m, 1H), 3.76 (s, 3H), 3.67−3.54 (m, 2H), 3.42−3.31 (m, 2H), 3.22−3.10 (m, 1H). LC-MS: RT = 4.58 min, $[M + H]^+$ = 357.0. Purity >95% by HPLC method A, RT = 4.02 min. Purity >95% by HPLC method B, RT = 6.09 min.

*5-Methoxy-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo-[d]thiazole Hydrochloride (**21**).* The compound was prepared as described for **14** from 2-chloro-5-methoxybenzo[d]thiazole[40] (202 mg, 1.01 mmol) and compound **11** (198 mg, 0.675 mmol). The *N*-Boc intermediate was purified by column chromatography (20−50% EtOAc in hexanes gradient), yielding 159 mg (52%) of an off-white solid. R_f = 0.20 in 70% EtOAc in hexanes. LC-MS: RT = 8.97 min, $[M + H]^+$ = 457.8. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 99 mg (72%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70−9.55 (br, 1H), 9.36−9.20 (br, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.22 (d, J = 4.4 Hz, 1H), 7.68 (dd, J = 8.4 and 1.2 Hz, 1H), 7.56−7.50 (m, 1H), 7.40 (dd, J = 8.6 and 4.6 Hz, 1H), 7.08 (d, J = 2.0 Hz, 1H), 6.75 (dt, J = 8.8 and 0.8 Hz, 1H), 4.81−4.73 (m, 1H), 4.59 (t, J = 9.0 Hz, 1H), 4.48 (dd, J = 10.2 and 6.2 Hz, 1H), 3.76 (s, 3H), 3.68−3.54 (m, 2H), 3.44−3.34 (m, 2H), 3.24−3.12 (m, 1H). LC-MS: RT = 4.28 min, $[M + H]^+$ = 357.5. Purity >95% by HPLC method A, RT = 4.07 min. Purity >95% by HPLC method B, RT = 5.47 min.

*6-Methyl-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]-thiazole Hydrochloride (**22**).* The compound was prepared as described for **14** from 2-chloro-6-methylbenzo[d]thiazole[41] (262 mg, 1.43 mmol) and compound **11** (175 mg, 0.953 mmol). The *N*-Boc intermediate was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 269 mg (42%, di-TFA salt) of a thick oil. LC-MS: RT = 9.60 min, $[M + H]^+$ = 441.8. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 75 mg (49%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76−9.60 (br, 1H), 9.42−9.26 (br, 1H), 8.35 (d, J = 3.6 Hz, 1H), 8.21 (d, J = 4.4 Hz, 1H), 7.62 (s, 1H), 7.54−7.48 (m, 1H), 7.44−7.34 (m, 2H), 7.12 (d, J = 8.0 Hz, 1H), 4.79−4.71 (m, 1H), 4.59 (t, J = 9.0 Hz, 1H), 4.50 (dd, J = 10.0 and 6.4 Hz, 1H), 4.10−4.02 (m, 1H), 3.68−3.54 (m, 2H), 3.44−3.32 (m, 2H), 3.24−3.10 (m, 1H), 2.34 (s, 3H). LC-MS: RT = 4.66 min, $[M + H]^+$ = 341.5. Purity >95% by HPLC method A, RT = 4.43 min. Purity >95% by HPLC method B, RT = 6.01 min.

*6-Chloro-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]-thiazole (**23**).* The compound was prepared as described for **14** from 2,6-dichlorobenzo[d]thiazole (104 mg, 0.511 mmol) and compound **11** (100 mg, 0.341 mmol). The *N*-Boc intermediate was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 93 mg (40%, di-TFA salt) of a thick oil. LC-MS: RT = 10.1 min, $[M + H]^+$ = 461.2. Deprotection gave 30 mg (52%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 2.8 Hz, 1H), 8.16 (dd, J = 4.8 and 1.2 Hz, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.49−7.44 (m, 1H), 7.41 (d, J = 8.8 Hz, 1H), 7.32 (dd, J = 8.6 and 4.6 Hz, 1H), 7.28 (dd, J = 8.8 and 2.4 Hz, 1H), 4.52 (t, J = 8.8 Hz, 1H), 4.40−4.32 (m, 1H), 4.28 (dd, J = 9.6 and 5.6 Hz, 1H), 3.76−3.67 (m, 1H), 3.40−3.28 (m, 1H), 3.13 (d, J = 12.0 Hz, 1H), 3.01−2.94 (m, 1H), 2.84 (dd, J = 12.8 and 4.0 Hz, 1H), 2.69 (dt, J = 12.4 and 3.2 Hz, 1H). LC-MS: RT = 5.00 min, $[M + H]^+$ = 361.1. Purity >95% by HPLC method A, RT = 4.77 min. Purity >95% by HPLC method B, RT = 6.30 min.

*2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)-6-(trifluoromethoxy)benzo[d]thiazole Hydrochloride (**24**).* The compound was prepared as described for **14** from 2-chloro-6-(trifluoromethoxy)benzo[d]thiazole[41] (250 mg, 0.985 mmol) and compound **11** (193 mg, 0.657 mmol). The *N*-Boc intermediate was purified by column chromatography (20−40% EtOAc in hexanes gradient), yielding 184 mg (55%) of the intermediate. R_f = 0.33 in 70% EtOAc in hexanes. LC-MS: RT = 10.3 min, $[M + H]^+$ = 511.9. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 109 mg (68%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36−8.96 (br, 2H), 8.31 (d, J = 2.8 Hz, 1H), 8.19 (dd, J = 4.8 and 1.2 Hz, 1H), 7.98 (d, J = 2.4 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.46 (ddd, J = 4.4, 2.8, and 1.2 Hz, 1H), 7.34 (dd, J = 8.8 and 4.8 Hz, 1H), 7.32−7.27 (m, 1H), 4.76−4.68 (m, 1H), 4.60−4.48 (m, 2H), 4.10−4.02 (m, 1H), 3.62 (dd, J = 12.8 and 2.8 Hz, 1H), 3.52 (d, J = 13.2 Hz, 1H), 3.38−3.28 (m, 2H), 3.11 (dt, J = 12.8 and 4.0 Hz, 1H). LC-MS: RT = 5.25 min, $[M + H]^+$ = 411.6. Purity >95% by HPLC method A, RT = 5.25 min. Purity >95% by HPLC method B, RT = 7.57 min.

*2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)-6-(trifluoromethyl)-benzo[d]thiazole Hydrochloride (**25**).* The compound was prepared as described for **14** from 2-chloro-6-(trifluoromethyl)benzo[d]-thiazole[41] (215 mg, 0.905 mmol) and compound **11** (177 mg, 0.603 mmol). The *N*-Boc intermediate was purified by column chromatography (20−40% EtOAc in hexanes gradient), yielding 97 mg (32%) of the intermediate. R_f = 0.35 in 75% EtOAc in hexanes. LC-MS: RT = 10.2 min, $[M + H]^+$ = 495.8. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 62 mg (74%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74−9.63 (br, 1H), 9.44−9.28 (br, 1H), 8.38 (d, J

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966−3983

= 2.8 Hz, 1H), 8.34 (s, 1H), 8.25 (dd, J = 4.8 and 1.4 Hz, 1H), 7.62 (d, J = 1.4 Hz, 2H), 7.56 (dd, J = 8.4 and 2.8 Hz, 1H), 7.44 (dd, J = 8.4 and 4.8 Hz, 1H), 4.88–4.82 (m, 1H), 4.64–4.55 (m, 2H), 4.20–4.12 (m, 1H), 3.74–3.64 (m, 1H), 3.59 (d, J = 13.2 Hz, 1H), 3.46–3.36 (m, 2H), 3.24–3.14 (m, 1H). LC-MS: RT = 5.17 min, $[M + H]^+$ = 395.6. Purity >95% by HPLC method A, RT = 5.10 min. Purity >95% by HPLC method B, RT = 7.25 min.

*6-Fluoro-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]-thiazole Hydrochloride (**26**).* The compound was prepared as described for **14** from 2-chloro-6-fluorobenzo[d]thiazole[40] (165 mg, 0.880 mmol) and compound **11** (172 mg, 0.587 mmol). The *N*-Boc intermediate was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 88 mg (22%, di-TFA salt) of the intermediate. LC-MS: RT = 9.27 min, $[M + H]^+$ = 445.7. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 35 mg (72%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78–9.62 (br, 1H), 9.44–9.28 (br, 1H), 8.35 (d, J = 2.8 Hz, 1H), 8.22 (dd, J = 4.8 and 1.2 Hz, 1H), 7.78 (dd, J = 8.8 and 2.8 Hz, 1H), 7.54–7.46 (m, 2H), 7.39 (dd, J = 8.6 and 4.2 Hz, 1H), 7.16 (dt, J = 9.2 and 2.8 Hz, 1H), 4.79–4.72 (m, 1H), 4.62–4.50 (m, 2H), 4.12–4.04 (m, 1H), 3.69–3.58 (m, 2H), 3.44–3.34 (m, 2H), 3.24–3.12 (m, 1H). LC-MS: RT = 4.47 min, $[M + H]^+$ = 345.5. Purity >95% by HPLC method A, RT = 4.20 min. Purity >95% by HPLC method B, RT = 5.93 min.

*4-Fluoro-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo[d]-thiazole Hydrochloride (**27**).* The compound was prepared as described for **14** from 2-chloro-4-fluorobenzo[d]thiazole[40] (144 mg, 0.767 mmol) and compound **11** (150 mg, 0.511 mmol). The *N*-Boc intermediate was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 92 mg (27%, di-TFA salt) of the intermediate. LC-MS: RT = 9.35 min, $[M + H]^+$ = 445.8. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 37 mg (71%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78–9.64 (br, 1H), 9.44–9.28 (br, 1H), 8.36 (d, J = 3.2 Hz, 1H), 8.23 (dd, J = 4.8 and 0.8 Hz, 1H), 7.67 (dd, J = 7.6 and 0.8 Hz, 1H), 7.57 (dd, J = 8.4 and 1.6 Hz, 1H), 7.40 (dd, J = 8.4 and 4.8 Hz, 1H), 7.22–7.10 (m, 2H), 4.84–4.77 (m, 1H), 4.63–4.53 (m, 2H), 4.16–4.08 (m, 1H), 3.72–3.56 (m, 2H), 3.44–3.34 (m, 2H), 3.24–3.14 (m, 1H). LC-MS: RT = 4.35 min, $[M + H]^+$ = 345.5. Purity >95% by HPLC method A, RT = 4.08 min. Purity >95% by HPLC method B, RT = 5.96 min.

*4,6-Difluoro-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)benzo-[d]thiazole Hydrochloride (**28**).* The compound was prepared as described for **14** from 2-chloro-4,6-difluorobenzo[d]thiazole[40] (158 mg, 0.767 mmol) and compound **11** (150 mg, 0.511 mmol). The *N*-Boc intermediate was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 85 mg (24%, di-TFA salt) of the intermediate. LC-MS: RT = 9.63 min, $[M + H]^+$ = 463.8. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 31 mg (63%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64–9.54 (br, 1H), 9.30–9.14 (br, 1H), 8.37 (d, J = 2.8 Hz, 1H), 8.24 (d, J = 3.2 Hz, 1H), 7.69 (dd, J = 8.0 and 1.6 Hz, 1H), 7.60–7.55 (m, 1H), 7.42 (dd, J = 8.0 and 4.4 Hz, 1H), 7.28 (dt, J = 10.6 and 2.8 Hz, 1H), 4.82–4.75 (m, 1H), 4.60–4.53 (m, 2H), 4.14–4.07 (m, 1H), 3.72–3.56 (m, 2H), 3.44–3.34 (m, 2H), 3.25–3.14 (m, 1H). LC-MS: RT = 4.65 min, $[M + H]^+$ = 363.5. Purity >95% by HPLC method A, RT = 4.36 min. Purity >95% by HPLC method B, RT = 6.39 min.

*2-(2-((Pyridin-3-yloxy)methyl)piperazin-1-yl)thiazolo[4,5-b]-pyridine Hydrochloride (**29**).* The compound was prepared as described for **14** from 2-chlorothiazolo[4,5-b]pyridine[41] (260 mg, 1.53 mmol) and compound **11** (300 mg, 1.02 mmol). The *N*-Boc intermediate was purified by column chromatography (50–100% EtOAc in hexanes gradient) and repurified by HPLC (5–50% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 62 mg (14%) of the intermediate. LC-MS: RT = 6.45 min, $[M + H]^+$ = 428.7. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 12 mg (22%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86–9.76 (br, 1H), 9.54–9.40 (br, 1H), 8.42 (d, J = 2.8 Hz, 1H), 8.40–8.34 (m, 2H), 8.28 (d, J = 4.4 Hz, 1H), 7.68–7.62 (m, 1H), 7.50 (dd, J = 8.4 and 4.8 Hz, 1H), 7.17 (dd, J = 7.4 and 5.0 Hz, 1H), 4.93–4.85 (m, 1H), 4.63 (d, J = 6.8 Hz, 2H), 4.24–4.15 (m, 1H), 3.76–3.40 (m, 4H), 3.26–3.14 (m, 1H). LC-MS: RT = 2.86 min, $[M + H]^+$ = 328.5. Purity >95% by HPLC method A, RT = 2.35 min. Purity >95% by HPLC method B, RT = 4.59 min.

*6-Chloro-2-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)thiazolo-[4,5-b]pyridine Hydrochloride (**30**).* The compound was prepared as described for **14** from 2,6-dichlorothiazolo[4,5-b]pyridine[41] (366 mg, 1.79 mmol) and compound **11** (262 mg, 0.893 mmol). The *N*-Boc intermediate was purified by column chromatography (50–75% EtOAc in hexanes gradient), yielding 88 mg (21%) of the intermediate. R_f = 0.39 in EtOAc. LC-MS: RT = 8.27 min, $[M + H]^+$ = 462.7. The material was deprotected and converted to the hydrochloride salt as described for **15**, yielding 74 mg (98%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80–9.67 (br, 1H), 9.52–9.35 (br, 1H), 8.45 (d, J = 2.4 Hz, 1H), 8.39–8.35 (m, 2H), 8.24 (dd, J = 4.6 and 1.0 Hz, 1H), 7.55 (dd, J = 8.4 and 2.0 Hz, 1H), 7.43 (dd, J = 8.4 and 4.8 Hz, 1H), 4.88–4.81 (m, 1H), 4.66–4.56 (m, 2H), 4.24–4.15 (m, 1H), 3.74–3.56 (m, 2H), 3.46–3.37 (m, 2H), 3.26–3.14 (m, 1H). LC-MS: RT = 4.16 min, $[M + H]^+$ = 362.5. Purity >95% by HPLC method A, RT = 3.87 min. Purity >95% by HPLC method B, RT = 5.81 min.

*Phenyl(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)methanone Dihydrochloride (**32**).* Benzoyl chloride (36 mg, 0.26 mmol) was added to a solution of **11** (72 mg, 0.25 mmol) in CH_2Cl_2 (4 mL). After stirring overnight, the material was concentrated under reduced pressure and purified by HPLC (10–95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 90 mg (71%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.45 min, $[M + H]^+$ = 398.2. Then 4 M HCl in 1,4-dioxane (6 mL, 24 mmol) was added to a solution of the intermediate in MeOH (1 mL). After stirring overnight, the reaction mixture was concentrated under reduced pressure, yielding 64 mg (98%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86–9.74 (br, 1H), 9.68–9.54 (br, 1H), 8.64–8.54 (m, 1H), 8.48–8.42 (m, 1H), 8.02–7.90 (br m, 1H), 7.86–7.74 (br m, 1H), 7.50–7.40 (m, 5H), 4.82–4.60 (br m, 2H), 4.60–4.24 (br m, 2H), 3.50–3.18 (m, 4H), 3.14–3.02 (m, 1H). LC-MS: RT = 3.05 min, $[M + H]^+$ = 298.1. Purity >95% by HPLC method A, RT = 2.79 min. Purity >95% by HPLC method B, RT = 3.26 min.

*(4-Chlorophenyl)(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)-methanone Dihydrochloride (**33**).* The compound was prepared as described for **32** from 4-chlorobenzoyl chloride (61 mg, 0.35 mmol) and **11** (85 mg, 0.29 mmol). The *N*-Boc intermediate was purified by column chromatography (40–60% EtOAc in hexanes gradient), yielding 84 mg (67%) of the intermediate. R_f = 0.31 in 100% EtOAc. LC-MS: RT = 9.18 min, $[M + H]^+$ = 432.1. The material was deprotected as described for **32**, yielding 78 mg (100%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84–9.74 (br, 1H), 9.66–9.52 (br, 1H), 8.68–8.48 (br m, 1H), 8.48–8.40 (m, 1H), 8.04–7.70 (br m, 2H), 7.58–7.46 (m, 4H), 4.84–4.56 (br m, 2H), 4.56–4.24 (br m, 2H), 3.54–3.15 (m, 4H), 3.14–3.02 (m, 1H). LC-MS: RT = 5.03 min, $[M + H]^+$ = 332.1. Purity >95% by HPLC method A, RT = 5.16 min. Purity >95% by HPLC method B, RT = 4.32 min.

*2-Phenyl-1-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)ethanone Dihydrochloride (**34**).* The compound was prepared as described for **32** from phenylacetyl chloride (40 mg, 0.26 mmol) and **11** (72 mg, 0.25 mmol). The material was purified by HPLC (10–95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 96 mg (74%) of the *N*-Boc intermediate TFA salt as a thick oil. LC-MS: RT = 7.75 min, $[M + H]^+$ = 412.2. The material was deprotected as described for **32**, yielding 63 mg (90%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66–9.42 (br, 2H), 8.63–8.40 (m, 2H), 7.88–7.74 (m, 2H), 7.33–7.18 (m, 5H), 5.06–4.98 (m, 1H), 4.82–4.74 (m, 1H), 4.64–4.53 (m, 1H), 4.52–4.39 (m, 2H), 3.83–3.76 (m, 1H), 3.50–3.42 (m, 2H), 3.32–3.10 (m, 2H), 2.86–2.64 (m, 1H). LC-MS: RT = 3.61 min, $[M + H]^+$ = 312.1. Purity >95% by HPLC

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966–3983

method A, RT = 3.35 min. Purity >95% by HPLC method B, RT = 3.74 min.

*2-(4-Chlorophenyl)-1-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)-ethanone Dihydrochloride (**35**).* The compound was prepared as described for **32** from 2-(4-chlorophenyl)acetyl chloride (69 mg, 0.37 mmol) and **11** (90 mg, 0.31 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 159 mg (93%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.46 min, $[M + H]^+$ = 446.1. The material was deprotected as described for **32** and was purified by HPLC (5−50% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 99 mg (83%) of the title compound as a white solid upon conversion to the dihydrochloride salt. The ^{1}H NMR indicates a 1.5:1 mixture of rotomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80−9.50 (br, 2H), 8.68−8.63 (br, 0.6H), 8.61−8.56 (br, 0.4H), 8.47 (d, J = 5.2 Hz, 1H), 8.07−8.01 (m, 0.6H), 7.96−7.90 (m, 0.4H), 7.88−7.79 (m, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.24 (appt. t, J = 9.2 Hz, 2H), 5.04−4.96 (m, 0.6H), 4.82−4.74 (m, 0.6H), 4.73−4.60 (m, 1.4H), 4.56−4.42 (m, 2.4H), 3.85−3.74 (m, 1H), 3.52−3.42 (m, 2H), 3.32−3.10 (m, 2H), 3.00−2.84 (m, 1H). LC-MS: RT = 4.28 min, $[M + H]^+$ = 346.1. Purity >95% by HPLC method A, RT = 4.06 min. Purity >95% by HPLC method B, RT = 4.81 min.

*2-Phenoxy-1-(2-((pyridin-3-yloxy)methyl)piperazin-1-yl)-ethanone (**36**).* The compound was prepared as described for **32** from 2-phenoxyacetyl chloride (68 mg, 0.37 mmol) and **11** (90 mg, 0.31 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 138 mg (84%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.85 min, $[M + H]^+$ = 428.2. The material was deprotected as described for **5** and was purified by HPLC (5−50% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 14 mg (16%) of the title compound as a thick oil upon free-basing with 1 N NaOH. The ^{1}H NMR indicates a 1:1 mixture of rotomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34−8.27 (m, 1H), 8.22−8.15 (m, 1H), 7.46−7.39 (m, 1H), 7.38−7.32 (m, 1H), 7.30−7.23 (m, 2H), 6.96−6.86 (m, 3H), 5.02−4.72 (m, 2H), 4.66−4.58 (m, 0.5H), 4.63 (t, J = 10 Hz, 0.5H), 4.38 (t, J = 11 Hz, 0.5H), 4.34−4.22 (m, 1H), 4.19−4.04 (m, 1H), 3.69−3.62 (m, 0.5H), 3.30−3.18 (m, 1H), 3.14−3.04 (m, 1H), 2.99−3.90 (m, 1H), 2.89−2.78 (m, 1H), 2.76−2.62 (m, 1H). LC-MS: RT = 3.66 min, $[M + H]^+$ = 328.1. Purity >95% by HPLC method A, RT = 3.42 min. Purity >95% by HPLC method B, RT = 3.86 min.

*N-Phenyl-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**37**).* Phenylisocyanate (41 mg, 0.34 mmol) was added to a solution of **11** (101 mg, 0.34 mmol) in CH_2Cl_2 (5 mL). After 1 h, the material was concentrated under reduced pressure and purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). The desired fractions were combined, brought to pH 12 with 1 N NaOH, and extracted with CH_2Cl_2 (3×). The combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure, yielding 115 mg (81%) of the *N*-Boc intermediate as a white solid. LC-MS: RT = 8.79 min, $[M + H]^+$ = 413.2. Then 4 M HCl in 1,4-dioxane (6 mL, 24 mmol) was added to a solution of the intermediate in MeOH (1 mL). After stirring overnight, the reaction mixture was concentrated under reduced pressure, yielding 105 mg (98%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82−9.73 (br, 1H), 9.70−9.58 (br, 1H), 8.96 (s, 1H), 8.69 (d, J = 2.4 Hz, 1H), 8.48 (d, J = 4.8 Hz, 1H), 8.11 (dd, J = 8.6 and 2.4 Hz, 1H), 7.88 (dd, J = 8.6 and 4.8 Hz, 1H), 7.44 (dd, J = 8.4 and 1.2 Hz, 2H), 7.24 (t, J = 7.8 Hz, 2H), 6.96 (dd, J = 7.8 and 1.2 Hz, 1H), 4.82−4.76 (m, 1H), 4.73 (t, J = 9.0 Hz, 1H), 4.58 (dd, J = 10.4 and 6.8 Hz, 1H), 4.22 (d, J = 14 Hz, 1H), 3.54−3.47 (m, 1H), 3.40−3.28 (m, 2H), 3.28−3.16 (m, 1H), 3.05−2.93 (m, 1H). LC-MS: RT = 5.28 min, $[M + H]^+$ = 313.1. Purity >95% by HPLC method A, RT = 4.89 min. Purity >95% by HPLC method B, RT = 3.57 min.

*N-(4-Chlorophenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**38**).* 4-Chlorophenylisocyanate (53 mg, 0.34 mmol) was added to a solution of **11** (101 mg, 0.34 mmol) in CH_2Cl_2 (5 mL). After 1 h, the material was concentrated under reduced pressure. LC-MS: RT = 9.54 min, $[M + H]^+$ = 447.1. Trifluoroacetic acid (1 mL) was added to a solution of the intermediate in CH_2Cl_2 (5 mL). After stirring overnight, the reaction mixture was concentrated under reduced pressure and was purified by HPLC (5−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 75 mg (52%) of the title compound as a white solid upon conversion to the dihydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70−9.62 (br, 1H), 9.58−9.45 (br, 1H), 9.10 (s, 1H), 8.64 (d, J = 2.8 Hz, 1H), 8.45 (d, J = 5.6 Hz, 1H), 8.02 (dd, J = 8.8 and 2.8 Hz, 1H), 7.82 (dd, J = 8.8 and 5.6 Hz, 1H), 7.49 (d, J = 6.8 Hz, 2H), 7.29 (d, J = 6.8 Hz, 2H), 4.82−4.74 (m, 1H), 4.67 (t, J = 9.0 Hz, 1H), 4.55 (dd, J = 10 and 6.8 Hz, 1H), 4.21 (br d, J = 14 Hz, 1H), 3.54−3.47 (m, 1H), 3.40−3.28 (m, 2H), 3.28−3.16 (m, 1H), 3.06−2.93 (m, 1H). LC-MS: RT = 4.38 min, $[M + H]^+$ = 347.1. Purity >95% by HPLC method A, RT = 4.19 min. Purity >95% by HPLC method B, RT = 4.92 min.

*N-(3-Chlorophenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**42**).* The compound was prepared as described for **37** from 3-chlorophenylisocyanate (25 mg, 0.16 mmol) and **11** (45 mg, 0.15 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 83 mg (97%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.32 min, $[M + H]^+$ = 446.9. The material was deprotected as described for **37**, yielding 62 mg (100%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68−9.59 (br, 1H), 9.55−9.42 (br, 1H), 9.17 (s, 1H), 8.63 (d, J = 2.4 Hz, 1H), 8.43 (d, J = 5.2 Hz, 1H), 8.02−7.95 (m, 1H), 7.79 (dd, J = 8.8 and 4.8 Hz, 1H), 7.63 (t, J = 2.4 Hz, 1H), 7.40 (ddd, J = 8.0, 2.0, and 0.8 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.01 (ddd, J = 8.0, 2.0, and 0.8 Hz, 1H), 4.92−4.84 (m, 1H), 4.65 (dd, J = 10.4 and 8.0 Hz, 1H), 4.55 (dd, J = 10.4 and 7.0 Hz, 1H), 4.25−4.18 (m, 1H), 3.54−3.47 (m, 1H), 3.38−3.28 (m, 2H), 3.28−3.18 (m, 1H), 3.06−2.94 (m, 1H). LC-MS: RT = 4.20 min, $[M + H]^+$ = 346.9. Purity >95% by HPLC method A, RT = 4.15 min. Purity >95% by HPLC method B, RT = 4.95 min.

*N-(3,4-Dichlorophenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**43**).* The compound was prepared as described for **37** from 3,4-dichlorophenylisocyanate (96 mg, 0.51 mmol) and **11** (150 mg, 0.51 mmol). The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 295 mg (97%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.96 min, $[M + H]^+$ = 481.1. The material was deprotected as described for **37**, yielding 208 mg (93%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68−9.59 (br, 1H), 9.55−9.44 (br, 1H), 9.35 (s, 1H), 8.63 (d, J = 2.4 Hz, 1H), 8.44 (d, J = 4.4 Hz, 1H), 8.02−7.97 (m, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.80 (dd, J = 8.6 and 5.0 Hz, 1H), 7.52−7.45 (m, 2H), 4.93−4.85 (m, 1H), 4.65 (dd, J = 10.0 and 8.0 Hz, 1H), 4.56 (dd, J = 10.0 and 7.0 Hz, 1H), 4.26−4.18 (m, 1H), 3.54−3.47 (m, 1H), 3.39−3.28 (m, 2H), 3.28−3.18 (m, 1H), 3.06−2.94 (m, 1H). LC-MS: RT = 4.87 min, $[M + H]^+$ = 381.0. Purity >95% by HPLC method A, RT = 4.76 min. Purity >95% by HPLC method B, RT = 6.16 min.

*2-((Pyridin-3-yloxy)methyl)-N-p-tolylpiperazine-1-carboxamide Dihydrochloride (**44**).* The compound was prepared as described for **37** from *p*-tosylisocyanate (43 mg, 0.32 mmol) and **11** (86 mg, 0.29 mmol). The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 149 mg (94%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.87 min, $[M + H]^+$ = 427.2. The material was deprotected as described for **37**, yielding 109 mg (98%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71−9.62 (br, 1H), 9.59−9.46 (br, 1H), 8.80 (s, 1H), 8.65 (d, J = 2.4 Hz, 1H), 8.45 (d, J = 4.4 Hz, 1H), 8.05−8.00 (m, 1H), 7.82 (dd, J = 8.8 and 5.6 Hz, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 4.88−4.81 (m, 1H), 4.68 (dd, J = 10.0 and 8.0 Hz, 1H), 4.52 (dd, J = 10.0 and 6.6 Hz, 1H), 4.23−4.15 (m, 1H), 3.54−3.46 (m, 1H), 3.38−3.27 (m, 2H), 3.26−3.16 (m, 1H), 3.05−2.92 (m, 1H), 2.23 (s, 3H). LC-MS: RT = 3.95 min, $[M + H]^+$ = 327.1. Purity >95% by HPLC method A, RT = 3.81 min. Purity >95% by HPLC method B, RT = 4.36 min.

*2-((Pyridin-3-yloxy)methyl)-N-(4-(trifluoromethyl)phenyl)-piperazine-1-carboxamide Dihydrochloride (**45**).* The compound was prepared as described for **37** from 4-(trifluoromethyl)-phenylisocyanate (47 mg, 0.25 mmol) and **11** (74 mg, 0.25 mmol).

dx.doi.org/10.1021/jm5004599 | *J. Med. Chem.* 2014, 57, 3966−3983

The material was purified by trituration with 1:1 CH_2Cl_2/hexanes, yielding 93 mg (77%) of the *N*-Boc intermediate as a white solid. LC-MS: RT = 8.78 min, $[M + H]^+$ = 481.9. The material was deprotected as described for **37**, yielding 86 mg (98%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67−9.58 (br, 1H), 9.54−9.42 (br, 1H), 9.37 (s, 1H), 8.61 (d, J = 2.8 Hz, 1H), 8.42 (d, J = 5.2 Hz, 1H), 7.99−7.94 (m, 1H), 7.77 (dd, J = 8.6 and 5.2 Hz, 1H), 7.68 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 8.8 Hz, 2H), 4.94−4.86 (m, 1H), 4.65 (dd, J = 10.0 and 7.6 Hz, 1H), 4.57 (dd, J = 10.0 and 7.2 Hz, 1H), 4.27−4.19 (m, 1H), 3.55−3.48 (m, 1H), 3.40−3.30 (m, 2H), 3.30−3.20 (m, 1H), 3.08−2.96 (m, 1H). LC-MS: RT = 4.85 min, $[M + H]^+$ = 381.6. Purity >95% by HPLC method A, RT = 4.68 min. Purity >95% by HPLC method B, RT = 5.79 min.

*N-(4-Isopropylphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**46**).* The compound was prepared as described for **37** from 4-isopropylphenylisocyanate (67 mg, 0.42 mmol) and **11** (122 mg, 0.42 mmol). The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 232 mg (98%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.89 min, $[M + H]^+$ = 455.9. The material was deprotected as described for **37**, yielding 135 mg (78%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70−9.60 (br, 1H), 9.56−9.44 (br, 1H), 8.80 (s, 1H), 8.64 (d, J = 2.8 Hz, 1H), 8.43 (d, J = 5.4 Hz, 1H), 8.04−7.98 (m, 1H), 7.80 (dd, J = 8.8 and 5.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 4.86−4.80 (m, 1H), 4.67 (dd, J = 10.0 and 8.4 Hz, 1H), 4.51 (dd, J = 9.6 and 6.8 Hz, 1H), 4.21−4.14 (m, 1H), 3.54−3.46 (m, 1H), 3.36−3.27 (m, 2H), 3.26−3.16 (m, 1H), 3.04−2.91 (m, 1H), 2.80 (hept, J = 6.8 Hz, 1H), 1.15 (d, J = 6.8 Hz, 6H). LC-MS: RT = 4.94 min, $[M + H]^+$ = 355.6. Purity >95% by HPLC method A, RT = 4.88 min. Purity >95% by HPLC method B, RT = 6.22 min.

*N-(4-Methoxyphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**47**).* The compound was prepared as described for **37** from 4-methoxyphenylisocyanate (76 mg, 0.51 mmol) and **11** (150 mg, 0.51 mmol). The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 251 mg (88%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.18 min, $[M + H]^+$ = 443.2. The material was deprotected as described for **37**, yielding 187 mg (99%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83−9.73 (br, 1H), 9.69−9.58 (br, 1H), 8.79 (s, 1H), 8.70 (d, J = 2.4 Hz, 1H), 8.48 (d, J = 5.2 Hz, 1H), 8.11 (dd, J = 8.8 and 2.4 Hz, 1H), 7.88 (dd, J = 8.8 and 5.2 Hz, 1H), 7.33 (d, J = 6.8 Hz, 2H), 6.83 (d, J = 6.8 Hz, 2H), 4.88−4.82 (m, 1H), 4.73 (dd, J = 10.0 and 8.4 Hz, 1H), 4.54 (dd, J = 9.6 and 6.4 Hz, 1H), 4.24−4.16 (m, 1H), 3.70 (s, 3H), 3.54−3.46 (m, 1H), 3.38−3.28 (m, 2H), 3.26−3.15 (m, 1H), 3.04−2.91 (m, 1H). LC-MS: RT = 3.40 min, $[M + H]^+$ = 343.1. Purity >95% by HPLC method A, RT = 5.09 min. Purity >95% by HPLC method B, RT = 3.79 min.

*(R)-N-(4-Methoxyphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride ((**R**)-**47**).* The compound was prepared as described for compound **47** from (***R***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88−9.78 (br, 1H), 9.74−9.60 (br, 1H), 8.81 (s, 1H), 8.71 (d, J = 2.4 Hz, 1H), 8.48 (d, J = 5.2 Hz, 1H), 8.12 (dd, J = 8.8 and 2.4 Hz, 1H), 7.89 (dd, J = 8.8 and 5.2 Hz, 1H), 7.33 (d, J = 6.8 Hz, 2H), 6.82 (d, J = 6.8 Hz, 2H), 4.89−4.82 (m, 1H), 4.74 (dd, J = 10.0 and 8.4 Hz, 1H), 4.55 (dd, J = 9.6 and 6.4 Hz, 1H), 4.24−4.16 (m, 1H), 3.70 (s, 3H), 3.53−3.46 (m, 1H), 3.38−3.26 (m, 2H), 3.25−3.14 (m, 1H), 3.04−2.91 (m, 1H). LC-MS: RT = 3.67 min, $[M + H]^+$ = 343.1. Purity >95% by HPLC method A, RT = 3.13 min. Purity >95% by HPLC method B, RT = 3.76 min.

*(S)-N-(4-Methoxyphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride ((**S**)-**47**).* The compound was prepared as described for compound **47** from (***S***)-**11**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70−9.62 (br, 1H), 9.58−9.46 (br, 1H), 8.74 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.06 (dd, J = 8.8 and 2.4 Hz, 1H), 7.84 (dd, J = 8.8 and 5.2 Hz, 1H), 7.32 (d, J = 6.8 Hz, 2H), 6.82 (d, J = 6.8 Hz, 2H), 4.86−4.80 (m, 1H), 4.69 (dd, J = 10.0 and 8.4 Hz, 1H), 4.51 (dd, J = 9.6 and 6.4 Hz, 1H), 4.22−4.14 (m, 1H), 3.70 (s, 3H), 3.53−3.44 (m, 1H), 3.36−3.27 (m, 2H), 3.26−3.16 (m, 1H), 3.04−2.92 (m, 1H). LC-MS: RT = 4.27 min, $[M + H]^+$ = 343.1. Purity >95% by HPLC method A, RT = 3.57 min. Purity >95% by HPLC method B, RT = 3.75 min.

*N-(3-Methoxyphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**48**).* The compound was prepared as described for **37** from 3-methoxyphenylisocyanate (24 mg, 0.16 mmol) and **11** (45 mg, 0.15 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 78 mg (91%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.47 min, $[M + H]^+$ = 442.9. The material was deprotected as described for **37**, yielding 57 mg (99%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74−9.65 (br, 1H), 9.61−9.49 (br, 1H), 8.90 (s, 1H), 8.65 (d, J = 2.8 Hz, 1H), 8.45 (d, J = 4.4 Hz, 1H), 8.06−8.00 (m, 1H), 7.82 (dd, J = 8.8 and 5.4 Hz, 1H), 7.17−7.11 (m, 2H), 7.06−7.00 (m, 1H), 6.54 (dt, J = 8.0 and 1.2 Hz, 1H), 4.90−4.83 (m, 1H), 4.68 (dd, J = 10.0 and 8.4 Hz, 1H), 4.55 (dd, J = 9.6 and 6.4 Hz, 1H), 4.23−4.16 (m, 1H), 3.70 (s, 3H), 3.54−3.46 (m, 1H), 3.38−3.28 (m, 2H), 3.27−3.17 (m, 1H), 3.06−2.93 (m, 1H). LC-MS: RT = 3.61 min, $[M + H]^+$ = 343.0. Purity >95% by HPLC method A, RT = 3.48 min. Purity >95% by HPLC method B, RT = 4.06 min.

*N-(2-Methoxyphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**49**).* The compound was prepared as described for **37** from 2-methoxyphenylisocyanate (38 mg, 0.26 mmol) and **11** (72 mg, 0.25 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 125 mg (92%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.84 min, $[M + H]^+$ = 443.2. The material was deprotected as described for **37**, yielding 91 mg (97%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66−9.55 (br, 1H), 9.54−9.40 (br, 1H), 8.60 (d, J = 2.4 Hz, 1H), 8.43 (d, J = 4.8 Hz, 1H), 8.03 (s, 1H), 7.97−7.91 (m, 1H), 7.77 (dd, J = 8.2 and 5.4 Hz, 1H), 7.58 (dd, J = 8.0 and 1.6 Hz, 1H), 7.07−7.00 (m, 2H), 6.92−6.86 (m, 1H), 4.80−4.72 (m, 1H), 4.65−4.53 (m, 2H), 4.17−4.10 (m, 1H), 3.79 (s, 3H), 3.52−3.44 (m, 1H), 3.38−3.20 (m, 3H), 3.07−2.94 (m, 1H). LC-MS: RT = 3.73 min, $[M + H]^+$ = 343.2. Purity >95% by HPLC method A, RT = 3.39 min. Purity >95% by HPLC method B, RT = 4.09 min.

*N-(2,4-Dimethoxyphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**50**).* The compound was prepared as described for **37** from 2,4-dimethoxyphenylisocyanate (46 mg, 0.26 mmol) and **11** (72 mg, 0.25 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 122 mg (84%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.53 min, $[M + H]^+$ = 473.2. The material was deprotected as described for **37**, yielding 89 mg (96%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67−9.58 (br, 1H), 9.53−9.41 (br, 1H), 8.64 (d, J = 2.8 Hz, 1H), 8.44 (d, J = 5.2 Hz, 1H), 8.02−7.93 (m, 2H), 7.80 (dd, J = 8.8 and 5.2 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.46 (dd, J = 8.8 and 2.8 Hz, 1H), 4.78−4.70 (m, 1H), 4.66 (appt t, J = 9.0 Hz, 1H), 4.50 (dd, J = 9.8 and 6.6 Hz, 1H), 4.17−4.07 (m, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.52−3.44 (m, 1H), 3.38−3.26 (m, 2H), 3.26−3.16 (m, 1H), 3.04−2.92 (m, 1H). LC-MS: RT = 3.45 min, $[M + H]^+$ = 373.1. Purity >95% by HPLC method A, RT = 3.48 min. Purity >95% by HPLC method B, RT = 4.11 min.

*N-(3-Chloro-4-methoxyphenyl)-2-((pyridin-3-yloxy)methyl)-piperazine-1-carboxamide Dihydrochloride (**51**).* The compound was prepared as described for **37** from 3-chloro-4-methoxyphenylisocyanate (76 mg, 0.42 mmol) and **11** (122 mg, 0.42 mmol). The material was purified by HPLC (10−90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 166 mg (68%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.87 min, $[M + H]^+$ = 477.8. The material was deprotected as described for **37**, yielding 119 mg (94%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65−9.56 (br, 1H), 9.50−9.38 (br, 1H), 8.92 (s, 1H), 8.62 (d, J = 2.8 Hz, 1H), 8.43 (d, J = 5.2 Hz, 1H), 8.02−7.95 (m, 1H), 7.78 (dd, J = 8.6 and 5.2 Hz, 1H), 7.57 (d, J = 2.4 Hz, 1H), 7.33 (dd, J = 9.0 and 2.4 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H), 4.86−4.79 (m, 1H), 4.64 (appt t, J = 9.0 Hz, 1H), 4.50 (dd, J = 10.2

dx.doi.org/10.1021/jm5004599 | *J. Med. Chem.* 2014, 57, 3966−3983

and 6.6 Hz, 1H), 4.22–4.15 (m, 1H), 3.79 (s, 3H), 3.54–3.46 (m, 1H), 3.36–3.26 (m, 2H), 3.26–3.16 (m, 1H), 3.05–2.92 (m, 1H). LC-MS: RT = 4.02 min, $[M + H]^+$ = 377.6. Purity >95% by HPLC method A, RT = 3.85 min. Purity >95% by HPLC method B, RT = 4.66 min.

*2-((Pyridin-3-yloxy)methyl)-N-(4-(trifluoromethoxy)phenyl)-piperazine-1-carboxamide Dihydrochloride (**52**).* The compound was prepared as described for **37** from 4-(trifluoromethoxy)-phenylisocyanate (65 mg, 0.32 mmol) and **11** (86 mg, 0.29 mmol). The material was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 161 mg (89%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.89 min, $[M + H]^+$ = 497.2. The material was deprotected as described for **37**, yielding 112 mg (90%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70–9.62 (br, 1H), 9.58–9.45 (br, 1H), 9.17 (s, 1H), 8.64 (d, J = 2.4 Hz, 1H), 8.44 (d, J = 5.2 Hz, 1H), 8.04–7.98 (m, 1H), 7.80 (dd, J = 8.6 and 5.2 Hz, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.25 (d, J = 8.8 Hz, 2H), 4.92–4.84 (m, 1H), 4.67 (appt t, J = 9.0 Hz, 1H), 4.55 (dd, J = 10.0 and 6.8 Hz, 1H), 4.26–4.18 (m, 1H), 3.54–3.47 (m, 1H), 3.40–3.28 (m, 2H), 3.28–3.18 (m, 1H), 3.06–2.94 (m, 1H). LC-MS: RT = 4.91 min, $[M + H]^+$ = 397.1. Purity >95% by HPLC method A, RT = 4.86 min. Purity >95% by HPLC method B, RT = 5.81 min.

*N-(4-Bromophenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**53**).* The compound was prepared as described for **37** from 4-bromophenylisocyanate (68 mg, 0.34 mmol) and **11** (101 mg, 0.34 mmol). The material was purified by HPLC (10–95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). The desired fractions were combined, brought to pH 12 with 1 N NaOH, and extracted with CH_2Cl_2 (3×). The combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure, yielding the *N*-Boc intermediate. LC-MS: RT = 9.69 min, $[M + H]^+$ = 491.1. The material was deprotected as described for **37** and was purified by HPLC (5–50% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). This gave 155 mg (97%) of the title compound as a white solid upon conversion to the dihydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77–9.68 (br, 1H), 9.64–9.51 (br, 1H), 9.14 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.47 (d, J = 5.2 Hz, 1H), 8.07 (dd, J = 8.8 and 2.4 Hz, 1H), 7.86 (dd, J = 8.8 and 5.2 Hz, 1H), 7.48–7.39 (m, 4H), 4.92–4.85 (m, 1H), 4.69 (dd, J = 10.0 and 8.0 Hz, 1H), 4.57 (dd, J = 9.6 and 6.8 Hz, 1H), 4.26–4.18 (m, 1H), 3.54–3.47 (m, 1H), 3.39–3.28 (m, 2H), 3.28–3.16 (m, 1H), 3.06–2.93 (m, 1H). LC-MS: RT = 4.48 min, $[M + H]^+$ = 391.0. Purity >95% by HPLC method A, RT = 4.35 min. Purity >95% by HPLC method B, RT = 5.28 min.

*N-(4-Bromo-3-methylphenyl)-2-((pyridin-3-yloxy)methyl)-piperazine-1-carboxamide Dihydrochloride (**54**).* The compound was prepared as described for **37** from 4-bromo-3-methylphenylisocyanate (88 mg, 0.42 mmol) and **11** (122 mg, 0.42 mmol). The material was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 165 mg (64%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.81 min, $[M + H]^+$ = 505.8. The material was deprotected as described for **37**, yielding 111 mg (87%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64–9.55 (br, 1H), 9.50–9.38 (br, 1H), 8.98 (s, 1H), 8.61 (d, J = 2.8 Hz, 1H), 8.42 (d, J = 4.8 Hz, 1H), 7.99–7.93 (m, 1H), 7.77 (dd, J = 8.6 and 4.8 Hz, 1H), 7.45–7.40 (m, 2H), 7.25 (dd, J = 8.8 and 2.4 Hz, 1H), 4.89–4.82 (m, 1H), 4.64 (appt t, J = 9.0 Hz, 1H), 4.53 (dd, J = 10.0 and 6.8 Hz, 1H), 4.23–4.15 (m, 1H), 3.54–3.47 (m, 1H), 3.38–3.28 (m, 2H), 3.28–3.17 (m, 1H), 3.06–2.94 (m, 1H), 2.28 (s, 3H). LC-MS: RT = 4.68 min, $[M + H]^+$ = 405.6. Purity >95% by HPLC method A, RT = 4.73 min. Purity >95% by HPLC method B, RT = 6.11 min.

*N-(4-Bromo-2-fluorophenyl)-2-((pyridin-3-yloxy)methyl)-piperazine-1-carboxamide Dihydrochloride (**55**).* The compound was prepared as described for **37** from 4-bromo-2-fluorophenylisocyanate (90 mg, 0.42 mmol) and **11** (122 mg, 0.42 mmol). The material was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 194 mg (75%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.50 min, $[M + H]^+$ = 509.8. The material was deprotected as described for **37**, yielding 141 mg (94%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70–9.62 (br, 1H), 9.57–9.44 (br, 1H), 8.82 (s, 1H), 8.63 (d, J = 2.4 Hz, 1H), 8.45 (d, J = 5.2 Hz, 1H), 8.04–7.98 (m, 1H), 7.81 (dd, J = 8.6 and 5.2 Hz, 1H), 7.53 (dd, J = 10.2 and 2.4 Hz, 1H), 7.42 (t, J = 8.8 Hz, 1H), 7.34 (dd, J = 8.8 and 2.4 Hz, 1H), 4.84–4.76 (m, 1H), 4.66 (appt t, J = 9.0 Hz, 1H), 4.55 (dd, J = 9.8 and 7.0 Hz, 1H), 4.18–4.10 (m, 1H), 3.54–3.45 (m, 1H), 3.40–3.28 (m, 2H), 3.28–3.18 (m, 1H), 3.06–2.94 (m, 1H). LC-MS: RT = 4.45 min, $[M + H]^+$ = 409.6. Purity >95% by HPLC method A, RT = 4.14 min. Purity >95% by HPLC method B, RT = 5.02 min.

*N-(Biphenyl-4-yl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**56**).* The compound was prepared as described for **37** from 4-isocyanatobiphenyl (78 mg, 0.40 mmol) and **11** (117 mg, 0.40 mmol). The material was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 210 mg (quantitative yield) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.92 min, $[M + H]^+$ = 489.2. The material was deprotected as described for **37**, yielding 126 mg (68%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65–9.56 (br, 1H), 9.52–9.38 (br, 1H), 9.02 (s, 1H), 8.62 (d, J = 2.8 Hz, 1H), 8.42 (d, J = 5.2 Hz, 1H), 8.00–7.95 (m, 1H), 7.77 (dd, J = 8.8 and 5.2 Hz, 1H), 7.63 (d, J = 6.8 Hz, 2H), 7.60–7.53 (m, 4H), 7.43 (t, J = 7.6 Hz, 2H), 7.31 (t, J = 7.6 Hz, 1H), 4.93–4.85 (m, 1H), 4.66 (appt t, J = 9.0 Hz, 1H), 4.55 (dd, J = 10.4 and 6.8 Hz, 1H), 4.26–4.19 (m, 1H), 3.56–3.48 (m, 1H), 3.40–3.30 (m, 2H), 3.30–3.20 (m, 1H), 3.08–2.96 (m, 1H). LC-MS: RT = 5.10 min, $[M + H]^+$ = 389.2. Purity >95% by HPLC method A, RT = 5.12 min. Purity >95% by HPLC method B, RT = 6.57 min.

*N-(4-Benzoylphenyl)-2-((pyridin-3-yloxy)methyl)piperazine-1-carboxamide Dihydrochloride (**57**).* The compound was prepared as described for **37** from 4-isocyanatobenzophenone (89 mg, 0.40 mmol) and **11** (117 mg, 0.40 mmol). The material was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 150 mg (60%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 8.43 min, $[M + H]^+$ = 517.2. The material was deprotected as described for **37**, yielding 80 mg (68%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67–9.58 (br, 1H), 9.54–9.43 (br, 1H), 9.42 (s, 1H), 8.60 (d, J = 2.8 Hz, 1H), 8.42 (d, J = 5.2 Hz, 1H), 7.99–7.94 (m, 1H), 7.77 (dd, J = 8.8 and 5.2 Hz, 1H), 7.72–7.62 (m, 7H), 7.58–7.53 (m, 2H), 4.96–4.89 (m, 1H), 4.68–4.56 (m, 2H), 4.27–4.20 (m, 1H), 3.57–3.48 (m, 1H), 3.40–3.31 (m, 2H), 3.31–3.21 (m, 1H), 3.08–2.96 (m, 1H). LC-MS: RT = 4.70 min, $[M + H]^+$ = 417.1. Purity >95% by HPLC method A, RT = 4.69 min. Purity >95% by HPLC method B, RT = 6.04 min.

*N-(2,3-Dihydrobenzofuran-5-yl)-2-((pyridin-3-yloxy)methyl)-piperazine-1-carboxamide Dihydrochloride (**58**).* The compound was prepared as described for **37** from 2,3-dihydro-1-benzofuran-5-yl isocyanate (55 mg, 0.34 mmol) and **11** (100 mg, 0.34 mmol). The material was purified by HPLC (10–90% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 157 mg (81%) of the *N*-Boc intermediate TFA salt as a white solid. LC-MS: RT = 7.28 min, $[M + H]^+$ = 455.2. The material was deprotected as described for **37** and was purified by HPLC (5–50% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). This gave 54 mg (45%) of the title compound as an off-white solid upon conversion to the dihydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72–9.63 (br, 1H), 9.60–9.48 (br, 1H), 8.69 (s, 1H), 8.66 (d, J = 3.2 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.09–8.03 (m, 1H), 7.83 (dd, J = 8.8 and 5.2 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.05 (dd, J = 8.8 and 2.0 Hz, 1H), 6.23 (d, J = 8.8 Hz, 1H), 4.86–4.79 (m, 1H), 4.69 (appt t, J = 9.4 Hz, 1H), 4.56–4.44 (m, 3H), 4.21–4.13 (m, 1H), 3.54–3.46 (m, 1H), 3.37–3.26 (m, 2H), 3.25–3.10 (m, 3H), 3.05–2.92 (m, 1H). LC-MS: RT = 3.87 min, $[M + H]^+$ = 355.1. Purity >95% by HPLC method A, RT = 3.48 min. Purity >95% by HPLC method B, RT = 3.82 min.

*tert-Butyl 4-(1H-Imidazole-1-carbonyl)-3-((pyridin-3-yloxy)-methyl)piperazine-1-carboxylate (**31**).* *N*,*N*-Carbonyldiimidazole (0.506 g, 3.12 mmol) was added to a solution of **11** (0.458 g, 1.56 mmol) in CH_2Cl_2 (15 mL). After stirring overnight, an additional portion of *N*,*N*-carbonyldiimidazole (1.27 g, 7.80 mmol) was added.

dx.doi.org/10.1021/jm5004599 | J. Med. Chem. 2014, 57, 3966–3983

After 4 h, the reaction mixture was diluted with EtOAc (30 mL) and washed with water (3 × 25 mL) and brine (25 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This gave 0.542 g (90% crude yield) of the title compound as a white foam. The material was used without further purification. LC-MS: RT = 5.87 min, $[M + H]^+$ = 388.1.

*Phenyl 2-((Pyridin-3-yloxy)methyl)piperazine-1-carboxylate Dihydrochloride (**39**).* Phenol (53 mg, 0.56 mmol), triethylamine (0.078 mL, 0.56 mmol), and **31** (109 mg, 0.28 mmol) were heated to 70 °C in acetonitrile (4 mL). After stirring overnight, Cs_2CO_3 (182 mg, 0.56 mmol) and phenol (53 mg, 0.56 mmol) were added and heating was continued for 1 h. Upon cooling to rt, the reaction mixture was filtered, concentrated under reduced pressure, and purified by HPLC (10−70% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). This gave 108 mg (73%) of the *N*-Boc intermediate TFA salt as a thick oil. LC-MS: RT = 8.54 min, $[M + H]^+$ = 414.2. Then 4 M HCl in 1,4-dioxane (6 mL, 24 mmol) was added to a solution of the intermediate in MeOH (1 mL). After stirring for 1 h, the reaction mixture was concentrated under reduced pressure, yielding 72 mg (91%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77−9.68 (br, 1H), 9.54−9.41 (br, 1H), 8.64 (br s, 1H), 8.43 (d, J = 4.4 Hz, 1H), 8.04−7.94 (m, 1H), 7.83−7.73 (m, 1H), 7.41 (t, J = 7.8 Hz, 2H), 7.25 (t, J = 7.4 Hz, 1H), 7.18−7.07 (m, 2H), 4.96−4.58 (m, 4H), 4.30−4.06 (m, 1H), 3.54−3.46 (m, 1H), 3.38−3.29 (m, 2H), 3.18−3.04 (m, 1H). LC-MS: RT = 3.89 min, $[M + H]^+$ = 314.1. Purity >95% by HPLC method A, RT = 3.61 min. Purity >95% by HPLC method B, RT = 4.22 min.

*4-Chlorophenyl 2-((Pyridin-3-yloxy)methyl)piperazine-1-carboxylate Dihydrochloride (**40**).* The compound was prepared as described for **39** from 4-chlorophenol (2 × 72 mg, 2 × 0.56 mmol) and **31** (109 mg, 0.28 mmol). The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient). The desired fractions were combined, brought to pH 12 with 1 N NaOH, and extracted with EtOAc (3 × 15 mL). The combined organics were washed with 1 N NaOH (2 × 30 mL) and brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This gave 68 mg (54%) of the *N*-Boc intermediate as a thick oil. LC-MS: RT = 9.24 min, $[M + H]^+$ = 448.1. The material was deprotected as described for **39**, yielding 63 mg (99%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93−9.83 (br, 1H), 9.68−9.54 (br, 1H), 8.69 (br s, 1H), 8.47 (d, J = 4.8 Hz, 1H), 8.10−8.03 (m, 1H), 7.90−7.80 (m, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.24−7.14 (m, 2H), 4.94−4.62 (m, 4H), 4.30−4.06 (m, 1H), 3.53−3.45 (m, 1H), 3.36−3.28 (m, 2H), 3.18−3.02 (m, 1H). LC-MS: RT = 4.66 min, $[M + H]^+$ = 348.1. Purity >95% by HPLC method A, RT = 4.40 min. Purity >95% by HPLC method B, RT = 5.35 min.

*4-Methoxyphenyl 2-((Pyridin-3-yloxy)methyl)piperazine-1-carboxylate Dihydrochloride (**41**).* *N*,*N*-Carbonyldiimidazole (90 mg, 0.56 mmol) was added to a solution of **11** (163 mg, 0.56 mmol) in CH_2Cl_2 (5 mL). After stirring overnight, the reaction mixture was concentrated under reduced pressure and dissolved in acetonitrile (10 mL). 4-Methoxyphenol (207 mg, 1.67 mmol) and triethylamine (1 mL) were added, and the reaction mixture was heated to 70 °C. After 6 h, the reaction mixture was diluted with EtOAc (50 mL) and was washed with 1 N NaOH (3 × 30 mL) and brine (30 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by HPLC (10−95% MeCN/0.1% TFA in H_2O/0.1% TFA gradient), yielding 155 mg (50%) of the *N*-Boc intermediate TFA salt as a colorless oil. LC-MS: RT = 8.46 min, $[M + H]^+$ = 444.2. The material was deprotected as described for **39**, yielding 106 mg (91%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82−9.73 (br, 1H), 9.58−9.46 (br, 1H), 8.66 (br s, 1H), 8.45 (d, J = 5.2 Hz, 1H), 8.06−7.98 (m, 1H), 7.86−7.78 (m, 1H), 7.10−7.00 (m, 2H), 6.92 (d, J = 8.8 Hz, 2H), 4.94−4.60 (m, 4H), 4.30−4.06 (m, 1H), 3.53−3.45 (m, 1H), 3.36−3.28 (m, 2H), 3.16−3.02 (m, 1H). LC-MS: RT = 4.07 min, $[M + H]^+$ = 344.1. Purity >95% by HPLC method A, RT = 3.76 min. Purity >95% by HPLC method B, RT = 4.69 min.

Rat PC12 Fluorescently Labeled α-Bungarotoxin Binding Assay. PC12 cells (ATCC, Manassas, Va.) were resuspended in binding buffer (PBS containing 1.0% FBS and 0.02% sodium azide) and added to a 96-well v-bottom plate at 0.08−1.5 × 10^5 cells per well. Each compound tested was diluted in binding buffer and added to the cells. Each sample contained 0.1% DMSO. Biotinylated α-bungarotoxin (Invitrogen Corporation, Carlsbad, California) was diluted in binding buffer and added to cells to yield a final concentration of 10 nM. An excess of unlabeled α-bungarotoxin was added to the nonspecific binding control at a final concentration of 1.5 μM. The samples were incubated at room temperature for 1 h. After incubation, the cells were washed one time with binding buffer to remove the unbound α-bungarotoxin. Phycoerythrin-labeled streptavidin (Becton-Dickinson Biosciences, San Jose, CA) was diluted in binding buffer and added to the cells for a final concentration of 1.0 μg/mL. The samples were incubated in the dark at room temperature for 15 min. The cells were washed once with binding buffer to remove the excess phycoerythrin-labeled streptavidin. The samples were then resuspended in binding buffer, and α-bungarotoxin binding was quantified by FACS analysis. Compounds were initially tested at 10 and 1 μM. Compounds showing 50% or greater inhibition of α-bungarotoxin binding at 10 μM were then tested in an eight-point IC_{50} assay. Briefly, compounds were 3-fold serially diluted and added to cells at the following concentrations: compounds showing greater than or equal to 50% inhibition at 10 μM and less than 75% inhibition at 1 μM were serially diluted from a concentration of 50 μM, compounds showing greater than 50% inhibition at 10 μM and between 70 and 95% inhibition at 1 μM were serially diluted from 10 μM, and last, compounds showing inhibition greater than 50% inhibition at 10 μM and greater than 95% inhibition at 1 μM were serially diluted from 1 μM. IC_{50} curves were then generated from the percent inhibition values at each of the eight concentrations.

Balb/c Mouse Ovalbumin Sensitization and Challenge Model of Allergic Lung Inflammation. Male, BALB/c mice, 6−8 weeks of age, were sensitized intraperitoneally with 10 μg of ovalbumin on days 1 and 14. On days 29, 30, and 31, the animals were challenged intranasally with 100 μg of ovalbumin. Test compounds were dissolved in saline and administered orally, starting 30 min prior to the three intranasal ovalbumin challenges. Groups of six mice were treated with either compound or vehicle control at the doses and intervals described in the figure legends. Sham groups of mice were sensitized with saline and challenged with allergen. The sham groups were included in the study to assess the effect of the sensitization-challenges procedure on the animals. Eight hours after the final ovalbumin challenge, mice were sacrificed and bronchoalveolar lavage (BAL) was performed. Microscope slides were prepared for each BAL sample and differential cell counts were performed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Binding assay data on panel of 55 receptors, ion channels, and transporters for compounds (***R***)-**18** and (***R***)-**47**. Activity inhibition data on panel of 11 enzymes for compounds (***R***)-**18** and (***R***)-**47**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Margreit J. Vervoordeldonk and Roger Papke for helpful discussions.

■ ABBREVIATIONS USED

BAL, bronchalveolar lavage; Boc, *t*-butoxycarbonyl; Cl, clearance; C_{max}, maximum plasma concentration; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; Cyp, cytochrome P450; DMF, *N*,*N*-dimethylformamide; *F*, oral bioavailability; FACS, fluorescence-activated cell sorter; FBS, fetal bovine serum; FEV1, forced expiratory volume in 1 s; hERG, human ether-à-go-go-related gene; IL, interleukin; IP, intraperitoneal; IV, intravenous; LPS, lipopolysaccharide; MW, molecular weight; nAChR, nicotinic acetylcholine receptor; NT, not tested; PBS, phosphate buffered saline; PK, pharmacokinetic; PMA, phosphomolybdic acid; PO, oral; SAR, structure−activity relationships; $T_{1/2}$, half-life; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TNF-α, tumor necrosis factor-α

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