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Letter

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Discovery of Indazoles as Potent, Orally Active Dual Neurokinin 1 Receptor Antagonists and Serotonin Transporter Inhibitors for the Treatment of Depression

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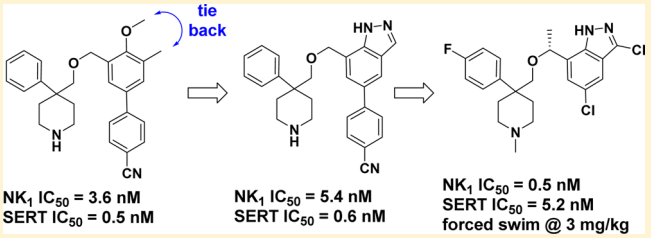
Supporting Information

ABSTRACT: Combination studies of neurokinin 1 (NK1) receptor antagonists and serotonin-selective reuptake inhibitors (SSRIs) have shown promise in preclinical models of depression. Such a combination may offer important advantages over the current standard of care. Herein we describe the discovery and optimization of an indazole-based chemotype to provide a series of potent dual NK1 receptor antagonists/serotonin transporter (SERT) inhibitors to overcome issues of ion channel blockade. This effort culminated in the identification of compound **9**, an analogue that demonstrated favorable oral bioavailability, excellent brain uptake, and robust in vivo efficacy in a validated depression model. Over the course of this work, a novel heterocycle-directed asymmetric hydrogenation was developed to facilitate installation of the key stereogenic center.



KEYWORDS: *Depression, neurokinin 1, serotonin transporter inhibitor, hERG, ion channels, asymmetric hydrogenation*

In antiquity, depression was thought to be due to a buildup of an excess of black bile, one of the four basic bodily humors.[1] Indeed, the word "melancholy" is derived from ancient Greek for "black bile" (*μέλαινα χολή, melaina chole*)[2] where it was linked with persistent fear and despondency. In modern times, the symptoms of depression have been blamed not on an excess of black bile, but on a deficiency of the neurotransmitter serotonin.

First described in 1967, the original serotonin hypothesis posited that reduced serotonin levels in the brain were the direct cause of depression, and that pharmacological restoration of serotonin levels would reduce symptomatology in patients.[3] This theory was based on clinical trials carried out by Coppen and co-workers, which showed that patients who received both a monoamine oxidase inhibitor (MAOI) and tryptophan, a brain-penetrant biogenic precursor to serotonin, showed more robust reductions in their depression than those given an MAOI alone.[4] While it was known that MAOIs increased the levels of monoamine neurotransmitters through the inhibition of enzymes that degrade them in the brain, the central role of serotonin was not elucidated until researchers combined these two serotonin-enhancing mechanisms into a single study. Since that time, it has become clear that genetic,[5] epigenetic,[6] environmental,[7] and neurobiological[8] factors all play a role in the development of depression and may lie upstream of the serotonergic effects.

First approved in the mid-1980s for the treatment of major depressive disorder (MDD), serotonin-selective reuptake inhibitors (SSRIs) inhibit reuptake of serotonin, increasing the synaptic concentrations of the neurotransmitter. Nearly 30 years since their introduction, SSRIs remain the bedrock of depression treatment, accounting for more prescriptions than all other antidepressant classes combined.[9] It should be noted that the shift from older MAOIs and tricyclic antidepressants to SSRIs has been driven more by their improved safety profile, rather than increased efficacy.[10] Despite their widespread use, only about 30% of patients achieve remission on SSRI-based therapy.[11] The utility of SSRIs is further limited by a delayed onset of action, requiring approximately 4 weeks of treatment prior to alleviation of symptoms.[12] As such, there remains a need to identify novel strategies to improve depression patient outcomes.

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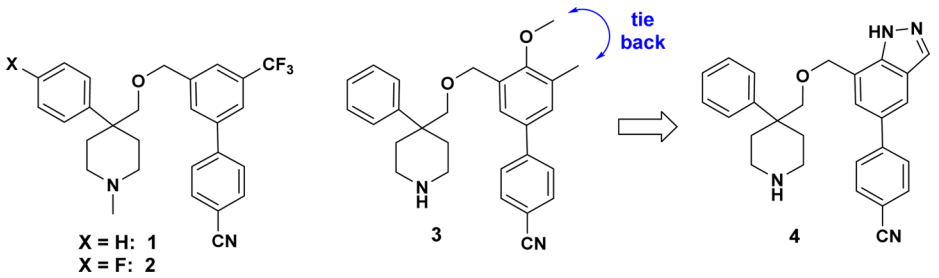

Figure 1. Genesis of the indazole chemotype.

Table 1. In Vitro Profiling of Compounds 1−9

	IC_{50}, nM				% inh @1 μM				
compd	hNK1	hSERT	SERT/NK1 ratio	MetStab h/r/m (% remain)[a]	hERG	Na	hPPB (% bound)	cLogP	LipE (SERT)
1	0.29	9.5	33	99/79/87		80		6.3	1.8
2	0.28	5.8	21	97/84/97	95	75	99.2	6.4	1.8
3	3.6	0.50	0.1	91/1/3			98.0	5.4	3.9
4	5.4	0.60	0.1	100/100/90		65	96.5	4.3	4.9
5	3.4	0.83	0.2					3.7	5.4
6	4.4	1.0	0.2					3.8	5.2
7	0.21	12	58					4.2	3.7
ent-7	200	39	0.2					4.2	3.2
8	0.11	5.4	48	65/5/35	36	73	96.6	4.6	3.7
9	0.46	5.2	11	88/81/96	90	90	99.1	5.2	3.1

[a]Percentage remaining after 10 min incubation with human (h), rat (r), or mouse (m) liver microsomes.

One such approach has been the combination of an SSRI with a neurokinin 1 (NK1) receptor antagonist. Interest in this combination was initiated by Froger and co-workers' study of SSRIs in NK1 knockout mice.[13] In this study, they demonstrated that wild-type and NK1 knockout mice show indistinguishable extracellular levels of serotonin. Upon acute administration of an SSRI, the levels of extracellular serotonin increased. However, the maximum SSRI-induced increase in NK1 knockouts was more than twice that observed in wild type animals. The authors further showed that the NK1 knockout mice demonstrated marked desensitization/downregulation of the 5-HT_{1A} autoreceptor. 5-HT_{1A} autoreceptors mediate negative feedback control on both the synthesis and release of serotonin.[14] Activation and desensitization of 5-HT_{1A} autoreceptors are known to be major mediators in the clinical efficacy of serotonergic therapies (busparone, SSRIs, MAOIs, serotonin precursor supplementation),[15] and the timing of desensitization mirrors the onset of clinical efficacy, suggesting the possibility for more rapid onset of action via combination therapy. Such a combination of an NK1 receptor antagonist with an SSRI, both targeting 5-HT_{1A} autoreceptor desensitization, may be as seminal to understanding and treating depression as Coppen's combination trials which first linked serotonin with depression and led to improved patient outcomes.

Indeed, multiple groups have shown that combination of subefficacious doses of NK1 antagonists with subefficacious doses of SSRIs leads to robust in vivo efficacy in validated rodent models of depression.[16] While approved medicines in both classes exist today, there are significant advantages to targeting dual activity in a single molecule.[17] First, this strategy necessarily aligns the absorption, distribution, metabolism, and excretion (ADME) properties of the two mechanisms, and thus gives rise to predictable relative inhibition of both targets across time and across bodily compartments in vivo. Additionally, a dual acting compound reduces patient pill burden without undertaking the often difficult and costly process of coformulating multiple active pharmaceutical ingredients. It was in this context that we undertook the development of a dual NK1 receptor antagonist/serotonin transporter (SERT) inhibitor.

In an earlier paper,[18] we reported the discovery of **1** (Figure 1) as a potent dual NK1 receptor antagonist and SERT inhibitor. This compound had excellent potency at both targets and a ratio consistent with our desired profile (SERT IC_{50}/NK1 IC_{50} ratio >10). The compound was orally bioavailable and highly brain penetrant (B:P = 2.5). Consistent with this profile, in ex vivo gerbil occupancy studies, **1** demonstrated the ability to maintain NK1 receptor saturation (>88% occupancy) while titrating the desired level of SERT occupancy (11−84%) via dose selection, suggesting the potential for antidepressant efficacy with reduced potential for SSRI-related side effects. Ultimately, on the basis of modest increases in SERT potency and metabolic stability, compound **2** became the lead compound from the biphenyl chemotype and was selected for more extensive *in vitro* profiling. Electrophysiology studies of **2** indicated that it was a potent inhibitor of both hERG and sodium channels. Screening of other leads from this class of compounds indicated that hERG inhibition was endemic to the series. There are three primary strategies routinely employed in attempts to reduce hERG binding: (1) formation of zwitterions to limit access to the transmembrane binding site, (2) reduction of the basicity of the amine moiety, and (3) reduction of log *P*.[19] As we were targeting effects in the CNS, we did not pursue a zwitterion-based strategy due to anticipated issues with brain uptake. We pursued pK_a modulation of the basic nitrogen, but, unfortunately, analogues which reduced the basicity of the piperidine moiety showed unacceptable losses in SERT potency (data not shown). Thus, we embarked upon a log *P*-based hERG mitigation strategy. It was recognized that

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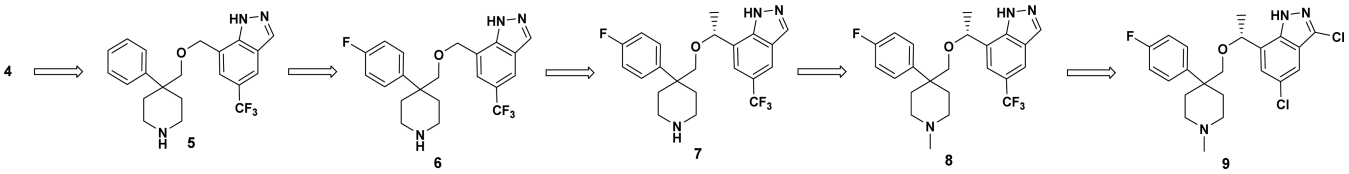

Figure 2. Evolution of indazole chemotype into lead **9**.

leads from this chemotype were highly lipophilic, with *N*-methylated derivatives (a requirement for favorable CNS penetration[18]) such as **1** and **2** having a cLogP > 6. As such, we sought to identify opportunities to introduce polar functional groups into this series. We drew inspiration from our biphenyl SAR where it was noted that that the methoxy group of **3** was well-tolerated, giving a compound with potent SERT and NK1 activity (albeit with reversed selectivity relative to the desired target profile, see Table 1). This result suggested the possibility of appending a heterocycle across C1 and C6 of the tetra-substituted phenyl group. We sought a heterocycle that would be electron donating at C1 (to mimic the C1-methoxy group of **3**) and electron-withdrawing at C6 (to mimic the trifluoromethyl of **2**), and felt that the indazole of **4** might achieve this goal. Furthermore, in choosing an indazole as our heterocyclic replacement, we anticipated a > 1 log unit drop in lipophilicity (cLogP) relative to related biphenyls. We were delighted to find that **4** had an essentially identical in vitro target profile to that of **3** (Table 1). It was also gratifying to find that the reduction in cLogP *in silico* correlated with reductions in plasma protein binding in vitro relative to **2** and **3** (Table 1).

With this lead in hand, we began to prepare analogues to develop SAR in this series with a particular interest in the identification of compounds with improved NK1 potency. The evolution of **4** to an optimized analogue is shown in Figure 2. The strategic decision was made to focus our efforts on the preparation of unsubstituted (NH) piperidines rather than the corresponding *N*-methylated derivatives for reasons of synthetic expediency. From earlier studies, we had found that, upon methylation, NK1 was only minimally impacted while SERT potency was generally within 3-fold of the unmethylated derivative. Unsubstituted piperidines that approached the desired in vitro profile (SERT/NK1 ratio >10, SERT < 10 nM) were subsequently converted to the corresponding *N*-methylpiperidine and characterized independently.

The 3,5-bistrifluoromethylphenyl motif is a privileged pharmacophore among NK1 receptor antagonists.[20,21] As the indazole of **4** had replaced the trifluoromethyl group of **2**, we replaced the cyanophenyl of **4** with this moiety. We were pleased to find that **5** had slightly improved NK1 potency and reduced lipophilicity. Over the course of the medicinal chemistry effort, the fluorophenyl of **2** overtook the unsubstituted phenyl of **1** as the preferred scaffold on the basis of trends toward increased liver microsomal stability and was thus installed to afford **6**. The critical advancement in NK1 receptor potency was achieved by incorporation of a methyl group in the benzylic position of the ether. Upon installation of a methyl group in the *R*-configuration, a 20× increase in NK1 receptor affinity was realized, while the SERT potency was reduced 10× to give a compound (**7**) which approached our targeted in vitro profile. It is of interest to note that while the *S*-methyl derivative (ent-**7**) is ∼1000× less potent at the NK1 receptor, SERT activity was reduced by only ∼3×. Previously, we demonstrated that unsubstituted piperidines showed poor brain uptake.[18] Therefore, the piperidine was methylated to afford **8**, a compound with exquisite potency at both targets and with SERT/NK1 ratio > 10.

In transforming from biphenyl **2** to indazole **8**, we had identified a molecule with a nearly identical in vitro target profile, while substantially reducing molecular weight (483 → 435) and cLogP (6.4 → 4.6). We also monitored lipophilic ligand efficiency[22] (LipE) as means of relating our lipophilicity to SERT potency. SERT-based LipE was chosen because, given our target profile of a SERT/NK1 ratio of >10, it was SERT potency that would ultimately impact dose selection. LipE also indicated substantial improvement, increasing by nearly two units (1.8 → 3.7). To measure the effect of reduced lipophility on our ion channel inhibition, **8** was examined in sodium and hERG patch clamp experiments. We were pleased to find that **8** had reduced inhibition at both channels. Unfortunately, liver microsomal stability was only moderate in human and low in rat and mouse. Suspecting that the most likely site of metabolism was at C3 of the indazole, a small library of C3-substituted indazoles was prepared. It was found that substitution of C3 of the indazole gave analogues with greatly improved microsomal stability, but with unacceptable losses in SERT potency (data not shown). By incorporating substitution at C3 and reoptimizing for SERT potency at C5, we identified **9**, a compound with high microsomal stability in all species. This compound also had an ideal target in vitro profile (NK1 = 0.45 nM, SERT = 5.2 nM; SERT/NK1 ratio = 11). Unfortunately, incorporation of the dichlorindazole led to a substantial increase in lipophilicity which was accompanied by a corresponding increase in hERG and sodium channel inhibition. A concentration response in patch clamp hERG and sodium channel indicated an $IC_{50} \sim 0.3\ \mu M$ at both targets. However, given its high microsomal stability and exquisite potency at both receptors, **9** was prioritized for advancement into efficacy studies in order to relate the ion channel liability to the exposures required to achieve in vivo efficacy.

The forced swim test (FST) is a validated model of antidepressant efficacy[23] which is based on the premise that rodents forced to swim in an inescapable environment will eventually become immobile (despair), restricting movements to only those which prevent drowning. After administration of the test compound, the animal is placed into an inescapable transparent tank of water and scored for the characteristic behavioral despair response (immobility). NK1 receptor radioligand binding studies have shown significant species differences in affinity for individual receptor antagonists. While mouse and rat receptor binding have proven poor predictors for human pharmacology, the gerbil has demonstrated good correlation with human.[24] Indeed, potency at gerbil NK1 and SERT were found to be in good agreement with human (gerbil IC_{50}s = 0.30 nM and 8.0 nM, respectively). As such, we employed gerbils in our forced swim test experiments. We were delighted to find that compound **9** was active in this depression model with a minimum effective dose (MED) of 3 mg/kg

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(Figure 3). In parallel with this experiment, exposure was obtained using satellite animals which indicated excellent brain

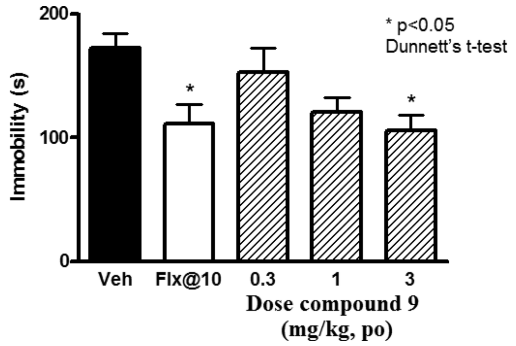


Figure 3. Gerbil forced swim test. Efficacy of compound **9** versus positive control (fluoxetine @ 10 mg/kg).

uptake (B:P ~ 7). At the MED, the total plasma concentration was 120 ± 53 nM and the total brain concentration of **9** was 809 ± 284 nM.

To assess the absolute and relative level of engagement at both targets, *ex vivo* occupancy experiments in gerbils were

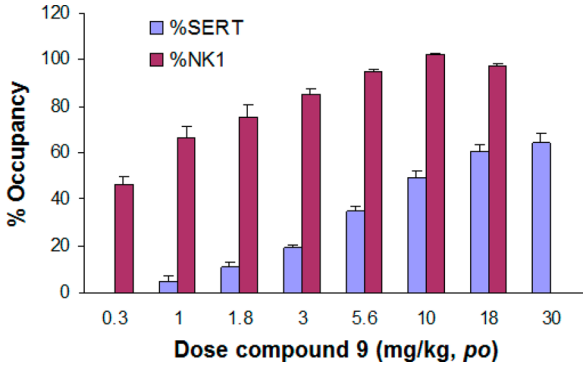


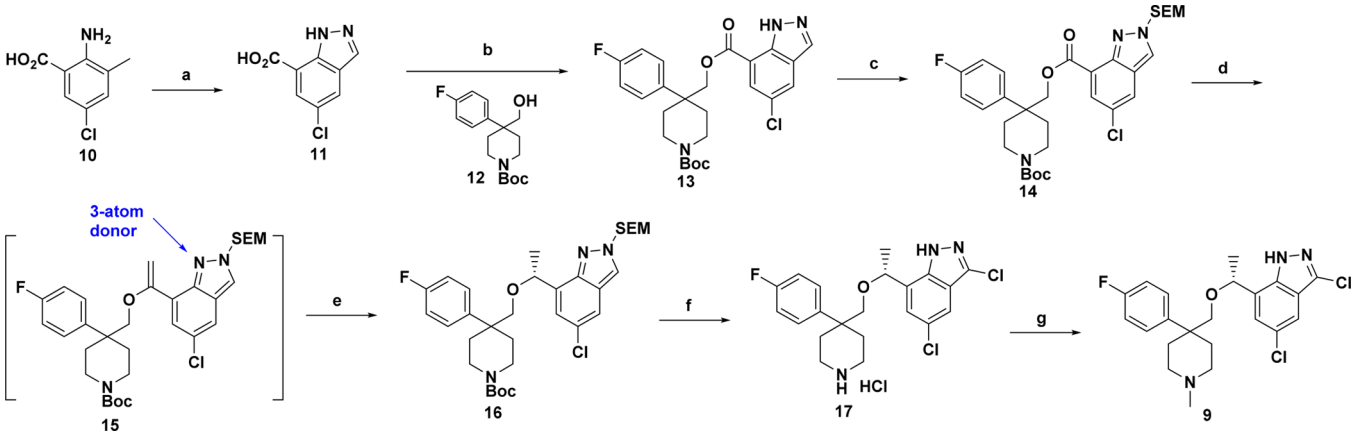
Figure 4. Neurokinin 1 receptor and serotonin transporter occupancy in gerbil.

performed with compound **9** across a range of doses (Figure 4). ^{125}I-substance P and ^{3}H-citalopram were used to assess NK1R and SERT occupancy, respectively. Appreciable occupancy was observed at both receptors at doses ≥1 mg/kg, and, as expected, the relative in vitro potency at each target translated to greater NK1 receptor occupancy at all doses. At the MED (3 mg/kg), NK1R and SERT occupancy were determined to be 88% and 20%, respectively (Figure 4).

The oral bioavailability of compound **9** was high (≥62%) across preclinical species (mouse, rat, dog, cyno). A human pharmacokinetic projection was prepared using simple allometry, yielding a prediction of low clearance (6.1 mL/min/kg) and moderate volume of distribution (4.9 L/kg). A 2 mg/kg dose (140 mg for a 70 kg human) anticipated 24 h target coverage (>120 nM plasma concentration at trough; MED in gFST) and a C_{max} = 260 nM (2.3 nM free) at steady state (attained ~48 h after initiation). This corresponded to >100× margins against both hERG and sodium ion channels. Follow-up studies in rabbit demonstrated that there were no effects on cardiac repolarization or cardiac conduction, indicating no hERG or sodium channel inhibition-related effects up to 10 mg/kg [C_{max} = 3.5 μM (~70 nM free)].[25] Having demonstrated acceptable margins against ion channel liabilities, a scale-up of compound **9** was initiated in support of toxicology studies (Scheme 1).

The synthesis of **9** began with diazotization of commercially available aniline **10**. Quenching of the diazonium with *t*-butyl thiol followed by base-induced cyclization afforded indazole **11** in nearly quantitative yield. The two major fragments were united via EDC-mediated esterification of carboxylic acid **11** with alcohol **12**.[25] Selective protection of N2 of the indazole was achieved using a combination of SEMCl and dicyclohexylmethylamine[26] to afford **14** as a single regioisomer. The key step of the synthesis was installation of the stereogenic methyl group. We opted to pursue a Petasis methylenation[27]/asymmetric hydrogenation strategy. The Petasis methylenation proceeded uneventfully to afford enol ether **15**. While we could find no precedent for the use of a heterocycle to direct an asymmetric hydrogenation, we reasoned that N1 of the indazole could serve as a three atom donor which, in conjunction with a chiral hydrogenation catalyst, might

Scheme 1. Optimized Route to Compound 9[a]



[a]Reagents and conditions: (a) (i) $NaNO_2$, HCl; (ii) *t*-BuSH; (iii) KO*t*-Bu (97%); (b) EDC, DMAP (81%); (c) SEMCl, *N*,*N*-dicyclohexylmethylamine (88%); (d) Petasis reagent, $PhCH_2C(CH_3)_2OAc$; (e) $Ru(OAc)_2$[(*R*)-*p*-TolBINAP], 100 psi H_2 (85%, two steps); (f) NCS then HCl (86%); (g) HCHO, $NaHB(OAc)_3$, DIEA (99%).

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facilitate delivery of hydrogen to a single face of the substrate. In collaboration with our process research group, a catalyst screen was performed using enol ether **15**. In all, 25 catalysts were screened in each of two solvents (methanol and dichloroethane). We were delighted to find that a Ru-based BINAP catalyst in DCE gave excellent conversion, affording the product in high optical purity (>99.8% ee). It is well-known that this class of catalysts require a coordinating group in close proximity to the site of hydrogenation in order to achieve high conversion and high enantioselectivity,[28] thus supporting our original hypothesis that the heterocycle would coordinate to the chiral catalyst to deliver hydrogen to a single face.

Having demonstrated proof of concept for the key step on a small scale, we initiated the synthesis of a 70 g lot of **9** by this route. While the Petasis methylenation had proceeded smoothly using a standard protocol on a small scale, upon scale-up, a substantial amount of an ethylidene byproduct[25] was observed. It was found that the byproduct could be suppressed by addition of 2-methyl-1-phenylpropan-2-yl acetate ($PhCH_2C(CH_3)_2OAc$) which served as a sacrificial ester, reacting only after enol ether **15** had been consumed.[29] After an extractive workup and a solvent switch from toluene to dichloroethane, the crude enol ether could be directly used in the asymmetric hydrogenation. This two-step Petasis/hydrogenation sequence was repeated successfully on batches that employed as much as 46 g of ester **14**. Installation of the second chlorine with NCS in acetic acid occurred with partial loss of the Boc group. A two-step one pot procedure was employed, adding HCl in isopropanol when chlorination was complete. This effected removal of both protecting groups and had the added benefit of allowing isolation of **17** as the crystalline HCl salt directly from the reaction mixture. Reductive amination using formalin and sodium triacetoxyborohydride completed the synthesis of **9**. This route was employed to successfully deliver 70 g of the compound of interest.

In toxicology studies, a single dose of **9** was well tolerated in mouse and rat up to 300 mg/kg (C_{max} = 29 and 9.1 μM, respectively).[25] In telemeterized rats, a single dose of 100 mg/kg did not alter blood pressure, heart rate, or locomotor activity (C_{max} = 7.3 μM). Unfortunately, upon repeat dosing for 2 weeks in rats, significant toxicity was observed. At 100 mg/kg, significant increases in aspartate- and alanine-aminotransferase (~5× and ~3× control, respectively) were observed. Histopathology revealed extensive tissue vacuolation. The frequency, severity, and the number of tissue types impacted increased with dose and was observed in all dose groups (10, 30, 100, and 300 mg/kg). The vacuolation was consistent with phospholipidosis which was subsequently confirmed via transmission electron microscopy.[25] At the lowest observed effect level (LOEL, 10 mg/kg), exposure was only 5−8× the projected human exposure and further progression of **9** was not pursued due to an insufficient margin for safety.

In conclusion, an analysis of the structure activity relationships of our dual NK1/SERT biphenyl chemotype suggested that a heterocyclic tie-back might be tolerated. This strategy was pursued to incorporate an indazole which reduced cLogP, mitigated ion channel inhibition, and reduced protein binding. Early leads from this series were optimized to afford **9**, a compound with favorable oral bioavailability, excellent brain uptake, and robust in vivo efficacy. Subsequent scale-up of **9** required development of a novel heterocycle-directed asymmetric hydrogenation to afford the key stereogenic center in high yield and excellent optical purity. While **9** demonstrated acceptable safety margins with respect to hERG and sodium channels, margins in a two-week rat toxicology study precluded further advancement of this novel NK1/SERT dual inhibitor.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.6b00337.

Detailed experimental procedures for the preparation of **9**, characterization data for compounds **1**−**8**, supplemental pharmacokinetic and toxicology data, and experimental details of biological assays (PDF)

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Author Contributions

A.P.D., J.J.B., J.E.M., and K.W.G. oversaw analogue design. A.P.D., G.O.T, and H.H. performed the synthesis of analogues. A.P.D., D.A.C., X.H., Y.H., T.R., J.-H.S., M.K.W., and H.Z. contributed the scale-up of compound **9** for toxicology studies. C.D.D., J.H., J.R., and S.J.T. contributed to the design and analysis of pharmacokinetic experiments. U.M.H. designed and analyzed toxicology experiments. R.K. performed NK1 and SERT binding assays. M.T.T. and N.J.L. oversaw the design and execution of in vitro and in vivo biological experiments. A.E.N. performed and analysed in vivo biology experiments. Y.-W.L. and R.L.P. generated occupancy data. A.P.D. wrote the manuscript with input from all authors.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CNS, central nervous system; FST, forced swim test; hERG, human ether-a-go-go-related gene; LipE, lipophilic ligand efficiency; MED, minimum efficacious dose; NCS, *N*-chlorosuccinimide; NK1, neurokinin 1; PPB, plasma protein binding; SAR, structure−activity relationship; SERT, serotonin transporter; SSRI, serotonin-selective reuptake inhibitor

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