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Discovery and Structure-activity Relationships of Nociceptin Receptor Partial Agonists that Afford Symptom Ablation in Parkinson's Disease Models

Uma Gayathri Kamakolanu,[1#] Michael E. Meyer,[1#] Dennis Yasuda,[1] Willma E. Polgar,[1] Matteo Marti,[2] Daniela Mercatelli,[3] Clarissa Anna Pisanò,[3] Alberto Brugnoli,[3] Michele Morari,[3] Nurulain T. Zaveri[1]*

[1] Astraea Therapeutics, 320 Logue Avenue, Suite 142, Mountain View, CA 94043, USA

[2] Department of Morphology, Surgery and Experimental Medicine, Section of Legal Medicine, University of Ferrara, Ferrara, Italy 44100.

[3] Department of Medical Sciences, Section of Pharmacology, University of Ferrara, Ferrara, Italy 44100.

[#] Both authors contributed equally to this work.

[*]**Address correspondence to:**

Dr. Nurulain T. Zaveri

Astraea Therapeutics,

320 Logue Avenue, Suite 142,

Mountain View, CA 94043, USA.

Tel: 650-254-0786

Email: nurulain@astraeatherapeutics.com

ABSTRACT

A novel series of C(3)-substituted-piperdinylindoles were developed as nociceptin opioid receptor (NOP) partial agonists to explore a pharmacological hypothesis that NOP partial agonists would afford a dual pharmacological action of attenuating Parkinson's Disease (PD) motor symptoms and development of levodopa-induced dyskinesias. SAR around the C-3 substituents investigated effects on NOP binding, intrinsic activity and selectivity and showed that, while the C(3)-substituted indoles are selective, high affinity NOP ligands, the steric, polar and cationic nature of the C-3 substituents affected intrinsic activity, to afford partial agonists with a range of efficacies. Compounds **4**, **5**, and **9** with agonist efficacies between 25-35% significantly attenuated motor deficits in the 6-OHDA-hemilesioned rat model of PD. Further, unlike NOP antagonists, which appear to worsen dyskinesia expression, these NOP partial agonists did not attenuate or worsen dyskinesia expression. The NOP partial agonists and their SAR reported here may be useful to develop nondopaminergic treatments for PD.

INTRODUCTION

Parkinson's Disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease affecting over 6 million individuals worldwide.[1] PD is clinically characterized as having classic motor symptoms (hypo/akinesia, rigidity, gait disturbance and resting tremor), as well as non-motor complications such as depression and cognitive decline.[2] The dopamine (DA) precursor levodopa (*L*-3,4-dihydroxyphenylalanine; L-DOPA) is the cornerstone of PD therapy, often given in combination with COMT and MAO inhibitors to extend its bioavailability and therapeutic action.[2] However, chronic L-DOPA therapy is associated with the eventual appearance (within 10 years in ~80% of patients) of motor fluctuations and dyskinesias (involuntary movements) that limit its clinical effectiveness and greatly reduce the quality of life of patients.[3] The pathogenic hallmark of PD is the neurodegeneration of dopamine producing neurons in the substantia nigra pars compacta leading to striatal dopamine depletion, which leads to the classic motor features. On the other hand, long-term manifestations of PD treatment, such as the levodopa-induced dyskinesias (LID) are the result of an irreversible brain sensitization to L-DOPA. There is only one marketed antidyskinetic drug, amantadine, a glutamate antagonist, which has poor and short-lasting clinical efficacy.[4] Current strategies to minimize LID involve reducing the L-DOPA dosage and combining it with a dopaminomimetic (e.g. DA receptor agonists) to maintain symptomatic benefit. Thus, newly developed drugs that can effectively treat PD symptoms, and delay or even prevent the development of dyskinesia, would provide significant relief to PD patients, and fill in a gap of unmet medical need in PD therapy.

Previous studies by Morari and co-workers have demonstrated the involvement of the nociceptin/orphanin FQ peptide receptor (NOP, known previously as ORL-1 (opioid receptor-

like-1 receptor)) in Parkinson's disease, particularly in development of motor symptoms.[5,6] The NOP receptor, a G protein coupled receptor (GPCR) is the fourth member of the opioid receptor family having close homology to the classical opioid receptors (mu, delta, kappa).[7] The endogenous ligand for the NOP receptor is the heptadecapeptide nociceptin/orphanin FQ (N/OFQ), which is similar to the endogenous kappa opioid peptide dynorphin, but has no binding affinity to the three classic opioid receptors. NOP activation by endogenous N/OFQ is implicated in many physiological functions and pathologies, including pain and analgesia, anxiety, learning and memory, as well as modulation of tolerance development and reward.[8] The NOP receptor is found throughout the brain, specifically, in brain cortical and subcortical areas, particularly in striatum, globus pallidus and substantia nigra (SNr) neurons.[9] Morari et al. have shown that endogenous N/OFQ contributes to the development of PD symptoms based on the following findings: 1) increased levels of N/OFQ found in the SNr following dopamine (DA) cell loss or impairment of DA transmission,[10,11] 2) NOP receptor antagonists reverse parkinsonian symptoms in neurodegenerative (6-OHDA hemilesioned rat, MPTP-treated mouse and macaque) and functional (reserpinized or haloperidol-treated animals) models of PD,[10,12,13] and 3) genetic deletion of the N/OFQ or NOP genes, or pharmacological blockade of the NOP receptor protects mice from the neurotoxic action of MPTP.[10,14] Mechanistic studies revealed that the antiparkinsonian action of NOP antagonists is accomplished through normalization of the imbalance between excitatory (Glu) and inhibitory (GABA) inputs to the nigro-thalamic neurons, generated by striatal DA deafferentation.[15,16] This suggests that NOP antagonists may provide a non-dopaminergic approach for the symptomatic and neuroprotective therapy of PD.

Morari and colleagues also showed that NOP receptor agonists attenuate the expression of abnormal involuntary movements (AIMs, a rodent correlate of LID) in dyskinetic rats and nonhuman primates challenged with L-DOPA, by acting in the striatum where, contrary to SNr, the N/OFQ tone is reduced and NOP receptors are up-regulated following DA cell loss.[17,18] This action can be dissociated from the typical motor inhibiting effects of NOP agonists since antidyskinetic doses were 100-fold lower than doses that caused hypolocomotion. Morari et al. showed that N/OFQ and NOP agonists specifically target dyskinesia pathways, preventing D1 receptor-mediated correlates of LID such as pERK phosphorylation and loss of depotentiation of synaptic plasticity in striatal GABA neurons.[17,18]

Based on the above evidence from NOP antagonists targeting PD symptoms by blocking nigral NOP receptors, and NOP agonists attenuating LID symptoms by stimulating up-regulated striatal NOP receptors, we hypothesized that NOP partial agonists could provide a balanced action in both areas affected in PD. A NOP partial agonist would be expected to function as an antagonist under conditions of high extracellular levels of endogenous N/OFQ (as in SNr) and alleviate parkinsonian motor dysfunction. On the other hand, in areas where the endogenous N/OFQ tone is low or absent and NOP receptors are upregulated (i.e. in striatum during LID development), NOP partial agonists would function as agonists. Based on this, we explored the discovery and structure-activity relationships of NOP partial agonists to identify suitable compounds to validate our hypothesis that NOP partial agonists may be a viable approach for PD and LID treatment, and to gain further insights into the role of the NOP-N/OFQ system in the pathophysiology of PD and dyskinesias.

RESULTS AND DISCUSSION

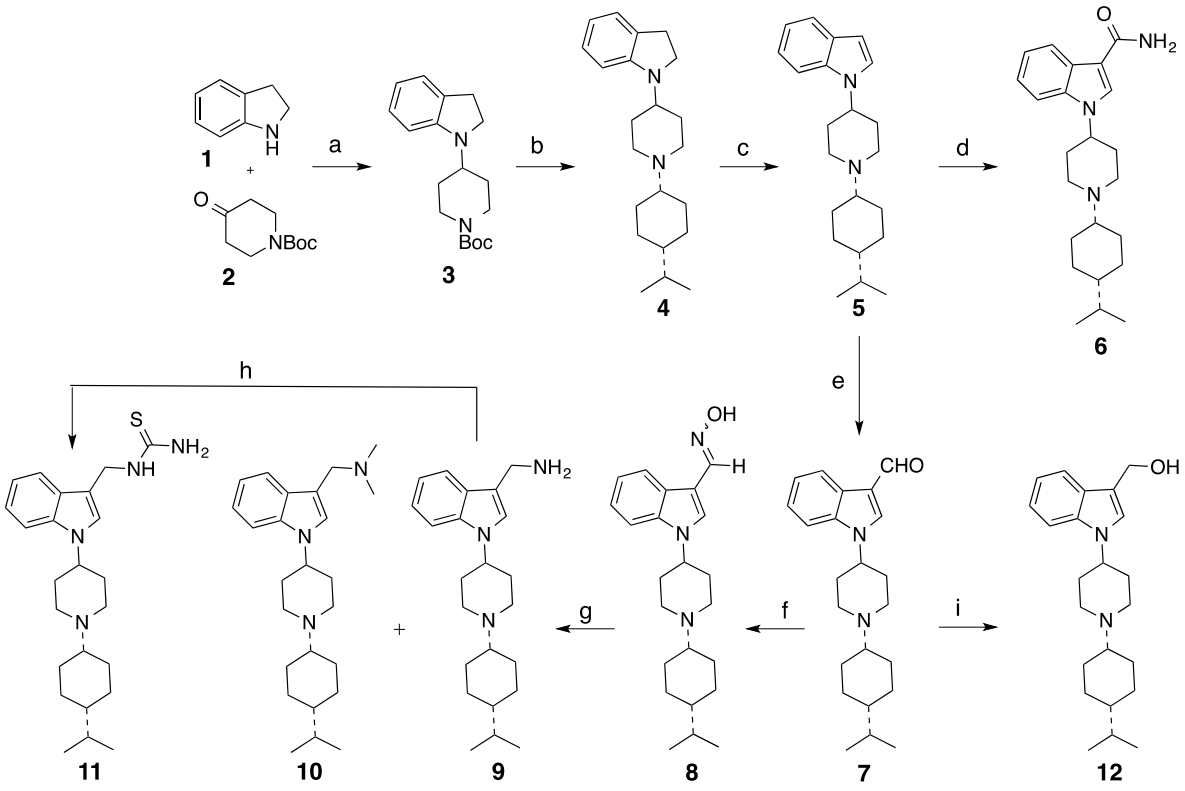
Chemistry. Zaveri et al previously disclosed NOP ligands with an indolinone motif (piperidinyl-indolinones) that provide both full and partial agonist efficacy and modest selectivity over the mu opioid receptor (MOP).[19-21] An SAR exploration was initiated with other similar bicyclic heterocycles, such as an indole and indoline ring in place of the indolinone moiety. This resulted in two new lead compounds **4** (AT-001) and **5** (AT-004), which were found to have partial agonist efficacy at NOP and >30-fold selectivity over the classical opioid receptors (Table 1). Further SAR investigation of these lead compounds to improve binding affinity and modulate intrinsic activity focused on the C(3) substituent of the indole ring is discussed below.

The synthesis of lead compounds indoline **4** and indole **5** is shown in Scheme I. Commercially available indoline **1** and N-Boc-4-piperidone **2** were subjected to standard reductive animation to provide intermediate **3** in 96% yield. Subsequent Boc removal, followed by a reductive alkylation of the piperidine nitrogen with 4-*i*Pr-cyclohexanone provided indoline **4** as a mixture of ca. 1.5:1 cis:trans isomers (with respect to the disubstituted cyclohexyl ring). The diastereomers could be separated via column chromatography to provide indoline **4** as a single diastereomer in 28% yield over two steps. Oxidation of **4** with MnO_2 in dichloromethane smoothly provided indole **5** in 79% yield.

To explore the SAR of different functionalities at the C-3 of the indole ring, several C-3 substituted indole analogs were synthesized using chemical transformations of indole **5**. Treating **5** with chlorosulfonylisocyanate ($ClSO_2NCO$) in MeCN provided C(3)-substituted amide **6** in modest yield. Aldehyde **7** was synthesized from indole **5** using Vilsmeier-Haack conditions (Scheme I). Conversion of aldehyde **7** to oxime **8** using standard conditions occurred readily in 96% yield. Oxime **8** was subsequently reduced using Ra (Ni) hydrogenation to afford

amine **9** (AT-035) in 78% yield, and dimethylamine **10** in low yield as a minor byproduct (Scheme I). In addition, reaction of amine **9** with benzoylthioisocyanate (BzNCS), followed by exposure to K_2CO_3 in MeOH readily provided thiourea **11** in moderate yield. Lastly, aldehyde **7** was smoothly converted to alcohol **12** via $NaBH_4$ reduction in 86% yield.

Scheme I

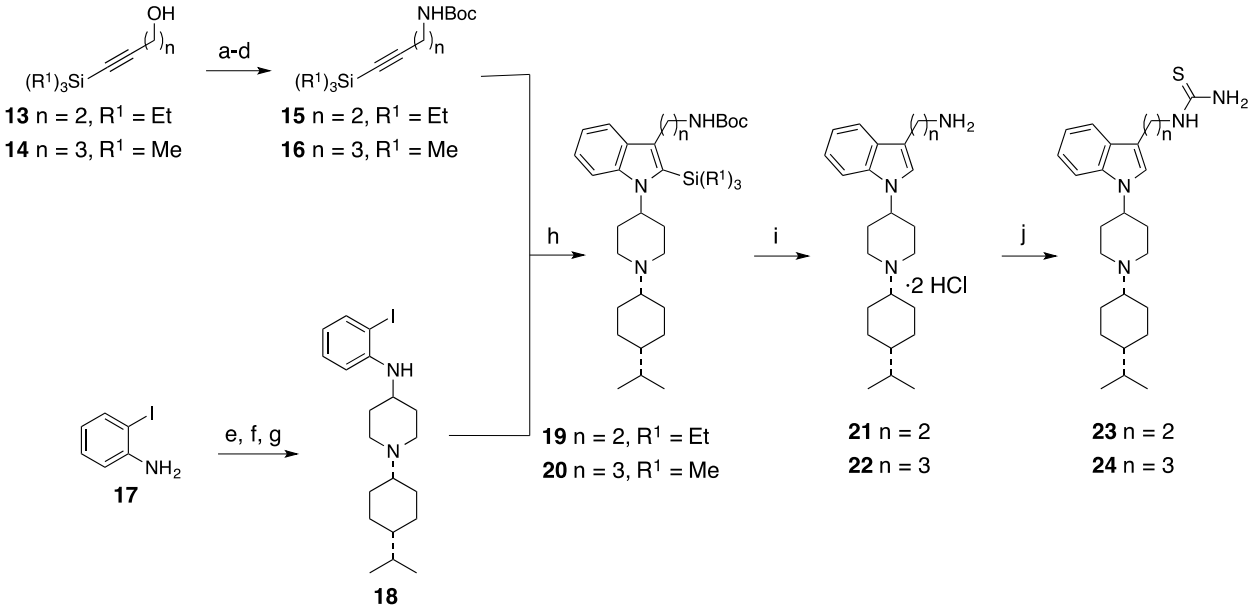


Reagents and Conditions: **(a)** AcOH, STAB, DCE, rt, 16 h, 96%; **(b)** i. TFA, CH_2Cl_2, 1h, ii. 4-*i*Pr-cyclohexanone, STAB, AcOH, DCE, rt, 1-2 days, 28% over 2 steps; **(c)** MnO_2, CH_2Cl_2, rt, 16 h, 79%; **(d)** $ClSO_2NCO$, MeCN, 0˚C, 30 min, 37%; **(e)** $POCl_3$, DMF, 0˚C, 1 h, 74%; **(f)** $NH_2OH{\cdot}HCl$, $NaOAc{\cdot}3H_2O$, EtOH:H_2O (2:1), 110 ˚C, 20 min, 96%; **(g)** H_2(g) (55 psi), 100 wt% Ra-Ni, MeOH, 16 h, 78% for **9**, 10-15% for **10**; **(h)** i. BzNCS, CH_2Cl_2, 0˚C, ii. K_2CO_3, MeOH, rt, 58%; **(i)** $NaBH_4$, EtOH, rt, 2 h, 86%.

To explore longer alkyl substitution at the indole C(3) position (e.g. **21-24**), the route was amended so that the indole ring was constructed using a Larock heteroannulation reaction

between a terminally silated alkyne and a N-substituted iodoaniline (Scheme II).[22] The N-Boc protected amino alkynes **15** and **16** were obtained from the corresponding alkynyl alcohols (**13** and **14**, respectively) in four straight-forward steps: 1) the alcohol was converted into a tosylate group, 2) S_N2 displacement of the tosylate group with phthalimide, 3) removal of the phthalimide group using hydrazine, and 4) Boc-protection of the amine.

Scheme II



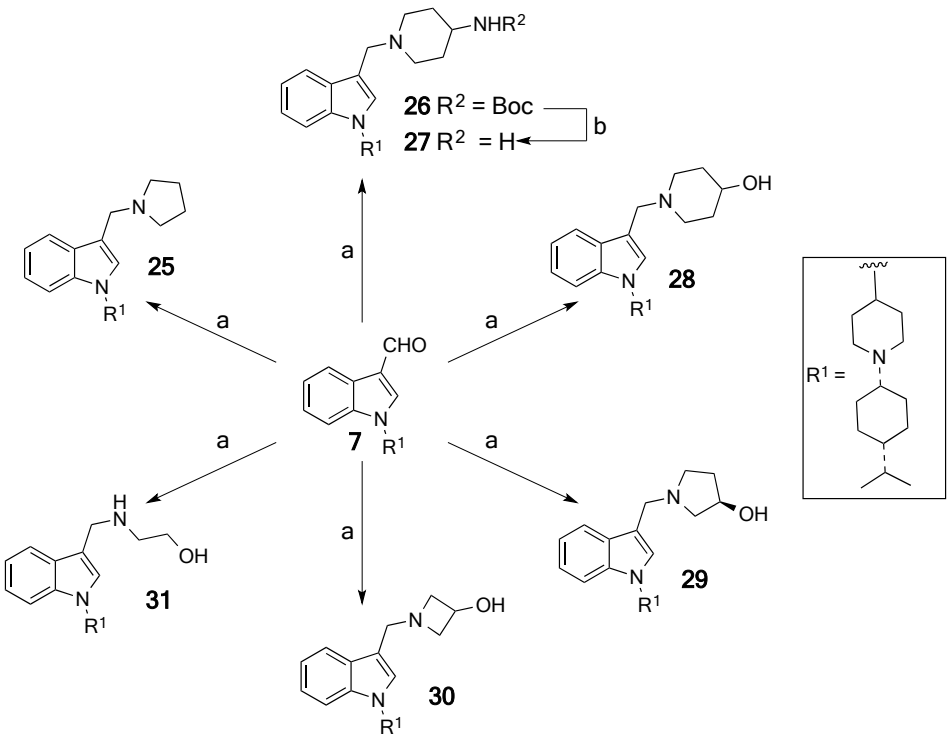
Reagents and Conditions: **(a)** TsCl, TEA, cat. DMAP, CH_2Cl_2, rt, 16 h, 93-99%; **(b)** phthalimide, K_2CO_3, DMF, 50˚C, 6 h, 67-94%; **(c)** NH_2NH_2, MeOH, rt, 16 h, 59-70%; **(d)** $(Boc)_2O$, cat. DMAP, THF, rt, 85-88%; **(e)** AcOH, STAB, DCE, rt, 16 h; **(f)** TFA, CH_2Cl_2, 0 ˚C⟶rt, 1 h; **(g)** 4-*i*Pr-cyclohexanone, AcOH, STAB, DCE, rt, 1-2 days, 39% over 3 steps; **(h)** alkyne **15** or **16**, cat. $Pd(OAc)_2$, LiCl, K_2CO_3, DMF, 102˚C, 2.5 h, 65-76%; **(i)** AcCl, MeOH, 0˚C⟶rt, then EtOAc, 78-80%; **(j)** i. BzNCS, CH_2Cl_2, 0˚C, ii. K_2CO_3, MeOH, rt, 47-50%.

The synthesis of the requisite iodoaniline **18** and the formation of the indole ring is shown in Scheme II. Commercially available 2-iodoaniline (**17)** was converted to intermediate **18** in a 3-step sequence consisting of 1) reductive amination with N-Boc-4-piperidone (**2**), 2)

removal of the Boc group, and 3) reductive amination of the piperidine nitrogen with 4-isopropylcyclohexanone in 39% yield over the three steps. At this stage, the key Larock heteroannulation reaction between iodoaniline **18**, and either alkyne **15** or **16**, proceeded in good yield to provide indole intermediates **19** and **20**, respectively. When indoles **19** and **20** were separately exposed to AcCl/MeOH conditions, clean removal of both the silyl group and the Boc moiety occurred to provide amines **21** and **22** *in situ* as bis HCl salts (Scheme II). Finally, ethyl-, and propyl-linked amines **21** and **22** each reacted with benzoylthioisocyanate (BzNCS) to give the respective ethyl-, and propyl-linked thioureas **23** and **24** in moderate yields (Scheme II).

Other C-3 substituents were introduced through further functionalization of aldehyde **7** via reductive amination with various nitrogen nucleophiles to provide C(3)-substituted cyclic and acyclic amines (**25**, **27**-**31)** in moderate to good yields (Scheme III).

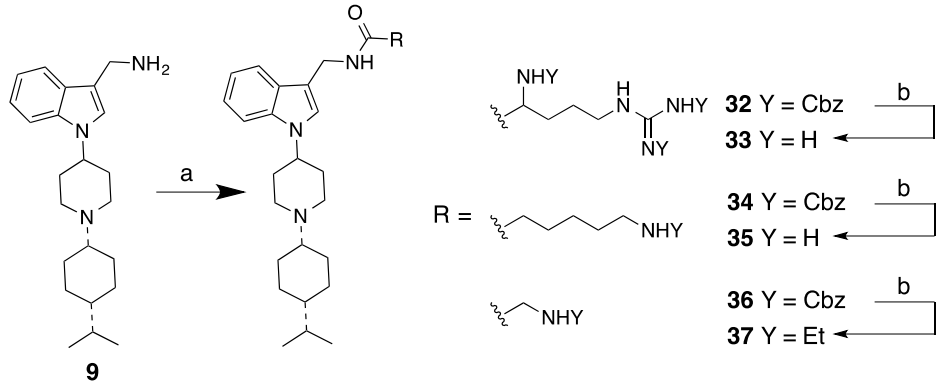
Scheme III



Reagents and Conditions: (a) amine, AcOH, STAB, DCE or MeOH, rt, 1-2 days, 22-98%; **(b)** TFA, CH_2Cl_2, 0˚C⟶rt, 20 min, 78%.

The synthesis of amides **33**, **35**, and **37** was accomplished in good yield from amine **9** via EDCI coupling with the corresponding N-Cbz-protected acid, followed by removal of the Cbz protecting group (Scheme IV). The use of the Cbz protecting group was critical in synthesizing amides **33**, **35**, and **37**, as the Boc-protected substrate degraded upon exposure to acidic conditions during deprotection.

Scheme IV



Reagents and Conditions: (a) HOBT, EDCI, TEA, N-Cbz acid, CH_2Cl_2, 0˚C, 67-88%; **(b)** H_2 balloon, 10 wt% Pd/C (10%), THF or EtOH, 3 h, 39-87%.

***In Vitro* Binding and Functional Efficacy.** The compounds were tested *in vitro* for binding affinity at the four opioid receptor subtypes in radioligand displacement assays in membranes from human NOP, MOP, KOP and DOP receptor-transfected chinese hamster ovary (CHO) cells, using methods we have described in detail previously.[21,23] Binding affinity was calculated as the binding constant Ki (nM), shown in Table 1. The intrinsic activity (functional efficacy) was determined using the [^{35}S]GTPγS binding assay in membranes prepared from the opioid receptor-transfected CHO cells, using methods we have previously published.[21,23,24] The intrinsic activities shown in Table 1 are reported as the % stimulation compared to that of the full agonist N/OFQ taken as 100%. The agonist potencies are reported as the EC_{50} (Table 1). All compounds showed weak binding affinity for the δ opioid receptor (DOP) and κ opioid receptor (KOP), and were not further tested for intrinsic activity at these receptors.

Compounds in Table 1 were designed to explore the SAR of the indole moiety on the N-substituted piperidine scaffold, the latter of which is a common motif in most NOP ligands.[25,26] SAR for small-molecule NOP partial agonists have not been extensively explored previously,

with respect to the effects of structure modification on NOP binding affinity, opioid selectivity or intrinsic efficacy.[27,28] To explore the SAR of the N-piperidinylindoles for high affinity NOP partial agonists, we focused on the C-3 substituent and maintained the N-isopropylcyclohexylpiperidinyl moiety in all the analogs synthesized, as this piperidinyl substituent has afforded high NOP binding affinity in other scaffolds we have investigated.[20,21,29]

INSERT TABLE 1 HERE

The C(3)-unsubstituted lead compounds indoline **4** and indole **5** show nanomolar affinity for NOP (10 nM), and weak affinity for the three classical opioid receptors (Table 1, entries 1 and 2), giving at least >40-fold selectivity for the NOP receptor. Both compounds show partial agonist efficacy at NOP in the [^{35}S]GTPγS functional assay. Indoline **4** showed a three-fold higher potency (EC_{50}) as an agonist at NOP than indole **5**. From the C(3)-substituted indoles bearing a polar substituent on a single methylene linker (compounds **9**, **10**, and **12**), primary amine **9** has high binding affinity (NOP Ki = 3 nM), ~20-fold selectivity versus MOP, and modest potency as a NOP partial agonist. Alcohol **12** and dimethylamino (tertiary amine substituent) **10** have 10-fold lower affinity than primary amine **9**, although alcohol **12** has comparable intrinsic activity to amine **9** whereas dimethylamine **10** showed significantly reduced agonist potency. We performed a chain-length SAR of amine **9**, to the ethyl-, and propyl-linked amines **21** and **22**, respectively. Both methyl- (**9**), and ethyl-linked (**21**) amines are optimal for NOP affinity and the increase in chain length to the ethyl-linked amine **21** also results in a 10-fold higher NOP selectivity compared to **9**. Functionally, the two compounds are both partial agonists at NOP, with similar potency. However, a further extension of the chain length to the propylamine **22** was not optimal for NOP binding.

There appears to be a steric size tolerance for a cationic amino group at the C(3) indole position. The pyrrolidinomethylene at C(3), as in compound **25**, showed high binding affinity for NOP (Ki 1.47 nM) and >200-fold selectivity versus the other opioid receptors. Interestingly, amine **25** also had high intrinsic activity, to a nearly full agonist (Table 1). However, a larger 4-amino-substituted piperidine moiety (as in compound **27**) resulted in a significant drop in affinity, although the 4-hydroxypiperidine analog **28** showed higher binding affinity than **27**. Interestingly however, 2-hydroxypyrrolidine **29** showed an even higher NOP binding affinity than hydroxypiperidine **28**, and an increase in intrinsic activity. It appears that pyrrolidine **25** and hydroxypyrrolidine **29** show higher agonist efficacy than the open-chain primary amines **9**, **21** and **22**, suggesting that these compounds may be binding in different orientations to the receptor due to steric reasons, differentially affecting the activation of the receptor towards higher intrinsic activity. Further contraction of the hydroxypyrrolidine ring of **29** to azetidine **30**, retains modest binding affinity at NOP (Ki = 23 nM), but shows very low agonist potency in the [^{35}S]GTPγS functional assay (EC_{50} = 1559 nM). In addition, acyclic alkyl amine **31**, an acyclic variant of hydroxypyrrolidine **29** showed weak binding affinity toward all four opioid receptors. When compounds **9**, **10**, **12**, **21**, and **22**, are compared to compounds **25** and **29,** it appears that cationic cyclic C(3) substituents, particularly pyrrolidinyl, show higher intrinsic activity (towards full agonist efficacy) compared to cationic open-chained C(3) substituents which show partial agonist activity.

Thiourea analogs **11**, **23**, **24** and amide analogs **33**, **35**, **37** were synthesized to investigate the importance of a cationic amine moiety at the C-3 substituent of the piperidinylindoles. The thiourea analogs **11**, **23**, and **24** showed only a modest (2-3-fold) drop in NOP binding affinity

and agonist potency, suggesting that it may be the hydrogen bonding or polar interactions of the C-3 substituent that are important for high affinity rather than a charged interaction.

Amides at the C-3 substituent containing an arginine unit (as in **33**), a lysine-like 6-aminohexanamide sidechain (as in **35**), or a ethylaminomethylamide (as in **37**) were also found to have only modest binding affinities (11–23 nM) at NOP, suggesting that a charged interaction of the guanidinium group in **33** or the amino group in **35** is likely not an important contributor to the binding affinity at NOP. Further, these compounds had poor agonist potencies and showed low intrinsic activity.

Compound **6**, containing an sp^2 hybridized C(3) substituent in the form of an amide, showed weak binding affinity for all the opioid receptors (Ki > 100 nM). However, the C(3)-oxime **8**, on the other hand, was found to be a high-affinity (Ki = 2 nM), NOP-selective (NOP/MOP = ca. 25 fold) potent full agonist.

Overall, our SAR showed that the C(3)-substituted piperidinylindoles afforded selective NOP agonists of varying efficacies, ranging from partial agonists (>20%) to nearly full agonist efficacy (>80%). It is possible that high intrinsic efficacy seen with C(3)-substituted indoles **8**, **25**, and **29** may arise from a different binding mode of these indoles (bearing a bulkier C(3) polar substituent, as for **25** and **29**) than the smaller alkyl-linked or unsubstituted indoles **5** or **9**. We have shown, using molecular dynamics simulations, that polar interactions between a NOP agonist ligand and NOP receptor residues at the extracellular end of the transmembrane helices and EL2 loop, result in agonist-induced reorganization of polar networks, during receptor activation.[30] Further SAR development supported by molecular docking and site-directed mutagenesis is needed to explain the observed NOP full agonism for compounds **8** and **25**.

To explore our hypothesis that NOP partial agonists would have dual efficacy in blocking PD symptoms and attenuating LID symptoms, four compounds were selected for further testing, based on the criteria of having high NOP binding affinity (Ki less than or equal to 10 nM) and partial agonist efficacy (20-35%) with agonist potency EC50 < 150 nM. Based on our in vitro pharmacological characterization, compounds **4**, **5**, **9** and **21** were selected for further in vitro ADME characterization and compounds **4**, **5** and **9** were advanced to in vivo pharmacokinetic evaluation for further investigation in vivo in PD models.

In Vitro ADME characterization and In vivo PK. Selected compounds were evaluated *in vitro* in ADME assays to characterize intestinal permeability, blood brain barrier permeability, and liver microsomal stability (Table 2). Indoline **4** and indole **5** show high permeability and low efflux in the Caco-2 intestinal permeability assay as well as in the MDR1-MDCK assay for BBB permeability (Table 2). Primary amines **9** and **21** showed lower permeability in the Caco-2 and BBB assay and high efflux in the BBB permeability assay. However, both primary amines **9** and **21** show excellent stability in rodent liver microsomes (Table 2), whereas indole **5** has a short half-life in rat liver microsomes. From the in vitro microsomal stability of the amine **9** compared to that of unsubstituted indole **5**, it appears that the unsubstituted C(3)-position in indole **5** may be a site of metabolic liability, and that the high permeability of indole **5**, which arises from its high lipophilicity, comes at the expense of solubility and metabolism.

INSERT TABLE 2 HERE

The plasma and brain concentrations of compounds **4**, **5** and **9** were determined in an in vivo pharmacokinetic study after subcutaneous administration to rats. As seen in Table 3, compounds **4** and **5** showed high total plasma and brain concentrations, with B/P ratios >6, suggesting high brain permeability of these compounds. Compound **9**, which showed poor BBB

permeability and high efflux in the in vitro BBB assay (Table 2) also showed a B/P ratio >1, although it showed overall lower concentrations than **4** or **5**, consistent with its lower permeability.

INSERT TABLE 3 HERE

Assessment of In vivo efficacy of NOP partial agonists in rat models of Parkinson's disease and levodopa-induced dyskinesia. NOP receptor partial agonists **4**, **5** and **9** were evaluated in vivo for their efficacy in improving parkinsonian motor disabilities in a rodent neurodegenerative model of PD, i.e. the 6-hydroxydopamine-induced hemilesioned rat model, that we have extensively used to characterize NOP antagonists.[10,15,16] The compounds were administered intraperitoneally (i.p.) in a range of doses.

Figure 1 shows that NOP partial agonists **4**, **5** and **9** attenuate parkinsonian disabilities in 6-OHDA hemilesioned rats. Parkinsonian motor deficits were assessed using three different measures of motor activity.[10,15,16,31] Acute administration of **5** (0.1-3 mg/kg, i.p.) improved motor deficits, partially reversing (~25%) the increase of immobility time (a measure of akinesia) at the contralateral (parkinsonian) paw in the bar test (Figure 1 panel D), and doubling stepping activity at the contralateral paw (a measure of akinesia/bradykinesia) in the drag test (Figure 1 panel E). Moreover, it enhanced by ~20%, the time spent on the rotarod (a measure of overall gait ability) (Figure 1B panel F). This profile is predictive of antiparkinsonian activity since it is replicated by L-DOPA[10, 16] and by NOP antagonists.[12,15,16] Compound **4** and **9** also showed similar patterns in improving parkinsonian motor deficits, although with some differences in efficacy in the three tests, at doses of 0.01-1 mg/kg (Figure 1, panels A-C and G-I), suggesting that the NOP partial agonists may actually be counteracting endogenous N/OFQ action at the NOP receptor, as predicted by our working hypothesis.

INSERT FIGURE 1 HERE

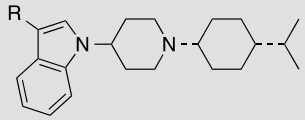
However, in the same dose ranges, all three compounds had no effect on the 'expression' of LID in fully dyskinetic rats challenged with L-DOPA, using methods previously reported by us (data not shown).[17,18] It is possible that the partial agonist efficacy of about 25-35% in all three compounds is too low to elicit an anti-dyskinetic effect seen with NOP full agonists that we have previously reported.[17,18] SAR studies to obtain suitable higher efficacy NOP partial agonists from the piperidinylindole and other novel NOP ligand scaffolds are underway to address this hypothesis and will be reported in due course.

CONCLUSIONS

In summary, we report the discovery and SAR of a novel series of 4-piperidinyl-1*H*-indoles as NOP receptor partial agonists. The SAR studies reported here represent the first systematic SAR study around NOP partial agonists and a novel NOP ligand scaffold, the piperidinyl-indoles. SAR analysis showed that the C(3)-substituted piperidinylindoles show significantly lower affinity for the classical opioid receptors mu, delta and kappa, resulting in selective NOP ligands with a range of intrinsic efficacies, ranging from partial agonists to nearly full agonists. Our SAR further indicates that size and polar nature of the C(3) substituent of the indole moiety influences the binding affinity and intrinsic activity of these ligands, such that smaller alkyl-linked C(3) substituents on the indole have partial agonist efficacy (e.g. compound **9**, **21**) whereas bulkier polar substituents have higher agonist efficacy (e.g. compound **25**), possibly arising from different binding modes favoring receptor activation. Selected NOP partial agonists **4**, **5** and **9** also reduced parkinsonian motor symptoms in the 6-OHDA-hemilesioned rat model but were ineffective in reducing LID symptoms at the same dose range. Together with our

previous studies, these results provide further evidence that the NOP receptor is a possible non-dopaminergic target for the symptomatic treatment of PD and that NOP receptor partial agonists are promising for development of new symptomatic PD therapies. The SAR of novel NOP partial agonists reported here will assist in future lead optimization efforts in this regard.

Table 1. Binding affinities and functional efficacies of C(3)-substituted piperidinylindoles at human opioid receptors



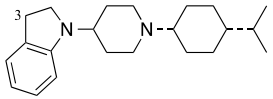
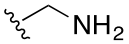
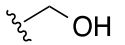
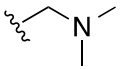
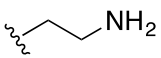
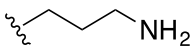
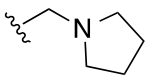
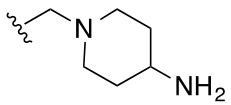
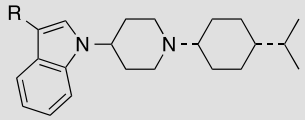
Compound number		Binding Affinities, K_i (nM)[a]				[S]^{35}GTPγS Functional Assay (nM)[b]			
		NOP	MOP	DOP	KOP	NOP		MOP	
						EC_{50}	% Stim	EC_{50}	% Stim
4		10.3 ± 0.03	604 ± 5	3070 ± 747	562 ± 150	54.5 ± 17.6	33.0 ± 2.8	ND	——
	R								
5	H	9.80 ± 0.86	376 ± 37	923 ± 413	1594 ± 57	160 ± 64	29.3 ± 9.5	232 ± 12.6[c]	16.3 ± 7.6[c]
9		3.27 ± 0.30	65.3 ± 2.4	>10K	1737 ± 172	121 ± 51.7	35.9 ± 5.7	410 ± 105	11.8 ± 2.7
12		44.7 ± 9.1	716 ± 21	>10K	>10K	117 ± 13.7	18.9 ± 0.70	ND	——
10		43.8 ± 7.4	504 ± 173	>10K	>10K	532 ± 185	28.1 ± 2.9	ND	——
21		2.27 ± 0.11	437 ± 118	>10K	2750 ± 383	139 ± 30.3	27.8 ± 7.2	ND	——
22		20.9 ± 7.6	46.2 ± 6.1	>10K	1320 ± 411	249 ± 9	18.6 ± 2.8	1764 ± 118	15.7 ± 3.2
25		1.47 ± 0.57	453 ± 107	>10K	461 ± 135	97.4 ± 7.4	87.9 ± 11.8	ND	——
27		216 ± 12	1308 ± 18	>10K	3456 ± 300	ND	——	ND	——

Table 1. Binding affinities and functional efficacies of C(3)-substituted piperidinylindoles at human opioid receptors



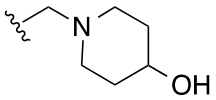
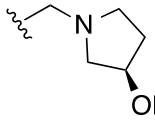
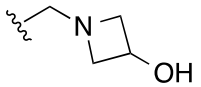
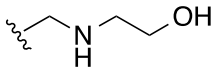
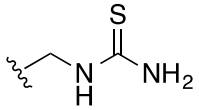
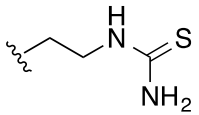
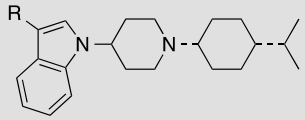
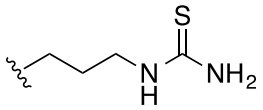
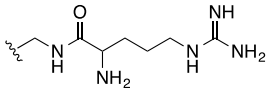
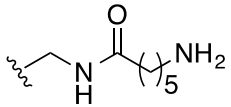
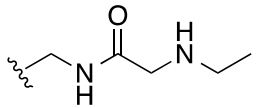
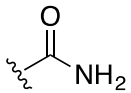
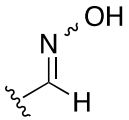
Compound number		Binding Affinities, K_i (nM)[a]				[S]^{35}GTPγS Functional Assay (nM)[b]			
		NOP	MOP	DOP	KOP	NOP		MOP	
						EC_{50}	% Stim	EC_{50}	% Stim
28		64.0 ± 21.3	>10K	6028 ± 652	3840 ± 1887	446 ± 130	10.6 ± 1.2	ND	——
29		9.46 ± 1.01	306 ± 77	>10K	>10K	575 ± 157	69.9 ± 12.1	ND	——
30		23.6 ± 3.1	>10K	>10K	>10K	1559 ± 398	34.3 ± 9.3	ND	——
31		136 ± 7.50	973 ± 211	>10K	1490 ± 52	ND	ND	ND	——
11		10.5 ± 1.25	71.3 ± 16.2	4067 ± 68	1328 ± 288	343 ± 18	51.2 ± 9.7	>10K	9.8 ± 2.1
23		24.4 ± 0.30	227 ± 50.0	>10K	81.0 ± 2.8[b]	>10K	5.6 ± 3.0	ND	——

Table 1. Binding affinities and functional efficacies of C(3)-substituted piperidinylindoles at human opioid receptors



Compound number		Binding Affinities, K_i (nM)[a]				[S]^{35}GTPγS Functional Assay (nM)[b]			
		NOP	MOP	DOP	KOP	NOP		MOP	
						EC_{50}	% Stim	EC_{50}	% Stim
24		31.8 ± 1.7	382 ± 129	>10K	830 ± 181	175 ± 23	34.5 ± 3.6	ND	——
33		11.0 ± 2.7	857 ± 109	>10K	674 ± 85	>10K	11.1 ± 1.5	ND	——
35		23.1 ± 8.5	949 ± 74	>10K	>10K	464 ± 124	37.7 ± 8.9	ND	——
37		15.9 ± 3.7	269 ± 12	>10K	1440 ± 342	277 ± 144	15.9 ± 4.9	ND	——
6		129 ± 41.4	453 ± 8	2712 ± 300	446 ± 24	ND	ND	ND	——
8		2.17 ± 0.35	56.4 ± 1.7	1690 ± 870	>10K	67.0 ± 11.0	85.9 ± 9.8	3199 ± 260	37.5 ± 2.9

[a] Binding affinities were determined using radioligand displacement assays performed in membranes of CHO cells stably expressing the human NOP, MOP, KOP and DOP receptors, and their respective ra-dioligands, [^{3}H]N/OFQ–NOP, [^{3}H]U69,593–KOP, [^{3}H]DAMGO–MOP and [^{3}H]DPDPE–DOP receptor. Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng−Prusoff equation. Each K_i value represents the arithmetic mean ± SEM from at least three independent experiments, each performed in triplicate. ND = not determined.

[b] Compounds with K_i values >100nM were not tested in functional assays (N.D.). At the DOP and KOP receptor, all compounds showed binding affinity K_i >100nM, hence functional efficacy at KOP and DOP not determined. Functional activity was determined by stimulation of [^{35}S]GTPγS binding to cell membranes, EC_{50} is the ligand concentration producing half maximal stimulation, % stimulation was obtained as a percentage of stimulation of the standard full agonists, N/OFQ (for NOP), and DAMGO (for MOP), which showed at least 2- to 5-fold stimulation over basal. Results are the means ± SEM for at least 3 independent experiments each performed in triplicate.

Table 2. *In Vitro* ADME data for indoles 4, 5, 9 and 21

	Caco-2 Bidirectional[a]			MDR1-MDCK Permeability[b]			Human Liver Microsomal Stability ($t_{1/2}$ min)	Rat Liver Microsomal Stability ($t_{1/2}$ min)	Mouse Liver Microsomal Stability ($t_{1/2}$ min)
	P_{app} (x 10^{-6}cm/s)		Efflux Ratio	P_{app}(x 10^{-6}cm/s)		Efflux Ratio			
	A–B	B–A	(Perm/Efflux)	A–B	B–A	(Perm/Efflux)			
4	30	25	0.8 (Hi/No)	26.8	33.8	1.3 (Hi/No)	ND	ND	2.8
5	24	19.1	0.8 (Hi/No)	28.8	15.2	0.5 (Hi/No)	5.3	5.8	
9	4.64	4.92	1.1 (Hi/No)	0.34	14.4	43 (Low/Hi)	>60	>60	
21	4.36	6.49	1.5 (Hi/No)	0.60	22.1	37 (Low/Hi)	ND	ND	>60

[a] (P_{app}>1.0x10^{-6} cm/s and efflux ratio<3, high perm/no efflux), [b] (P_{app} A–B >3.0 and efflux ratio <3 = high BBB permeability, efflux ratio>3 = high efflux). These experiments were conducted according to methods described briefly in the Experimental section.

Table 3. In vivo pharmacokinetics for selected indoles 4, 5 and 9 after s.c. administration to Sprague Dawley rats.[a]

	Dose (mg/kg)	Plasma				Brain[c]				B/P Ratio (at C_{max})
		C_{max} (nM)	T_{max} (h)	AUC_{last} (nM.h)[b]	t ½ (h)	C_{max} (nM)	T_{max}(h)	AUC_{last} (nM.h)	t ½ (h)	
4	3 (s.c.)	456	0.5	1246	2.13	3069	0.5	6695	2.71	6.73
5	3 (s.c.)	626	1	1960	2.19	4166	0.5	10104	2.43	6.65
9	3 (s.c.)	529	0.5	1004	2.61	956	4.00	6129	10.8	1.81

[a] These experiments were conducted according to methods described briefly in the Experimental section.

[b] Timepoint for AUC_{last} is 8h

[c] Concentrations represent total brain concentrations

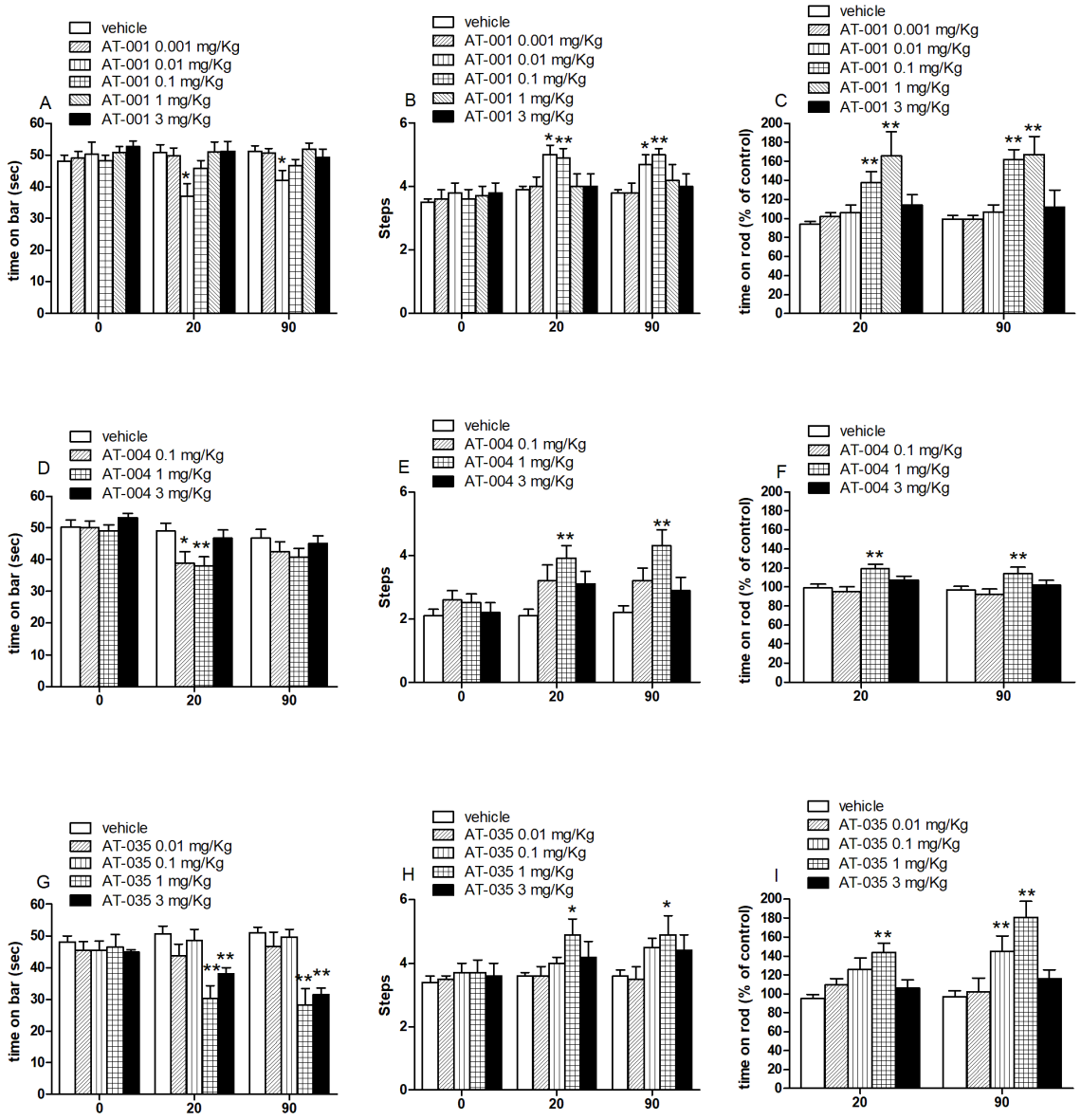


Figure 1. Effect of NOP partial agonists 4 (AT-001), 5 (AT-004) and 9 (AT-035) on parkinsonian disabilities in the 6-OHDA hemilesioned rat model of PD. Acute systemic administration of **4, 5** and **9** in a range of doses (*panels A–C*, **4**, AT-001, 0.001-3 mg/kg, i.p.) (*panels D–F*, **5**, AT-004, 0.1-3 mg/kg, i.p.) or (*panels G–I*, **9**, AT-035, 0.01 – 3 mg/kg, i.p.) attenuated motor deficits in three behavioural measures, (A) the bar test (*left graphs*), (B) the drag test (*middle graphs*) and (C) rotarod test (*right graphs*). Data are means ± SEM of 6-7 (AT-

001 and AT-035) or 17-23 (AT-004) determinations per group. AT-001 data (panels A-C) are means of 17-23 mice per group, AT-004 data (panels D-F) are means of 6-12 mice per group, and AT-035 data (G-I) are means of 6-7 mice per group. Essential statistical values: treatment effect *panels A-C* ($F_{5,2}$=4.60, p=0.0007, $F_{5,2}$=4.52, p=0.0008, $F_{5,1}$=13.72, p<0.0001, respectively), *panels D-F* ($F_{3,2}$=4.39, p=0.0051, $F_{3,2}$=8.45, p<0.0001, $F_{3,1}$=7.59, p<0.0001, respectively), *panels G-I* ($F_{4,2}$=14.31, p<0.0001, $F_{4,2}$=3.79, p=0.0071, $F_{4,1}$=11.51, p<0.0001, respectively) **P* < 0.05, ***P* < 0.01 different from vehicle (saline + 5% Tween 80 and 1% DMSO).

EXPERIMENTAL SECTION

CHEMISTRY METHODS

Thin layer chromatography was performed on Analtech silica gel GF 250 micron TLC plates. The plates were visualized with a 254 nm UV light and iodine. Flash chromatography was carried out on F60 silica gel, 43-60 ⌈m (230-400 mesh), 60 Å (Silicycle SiliaFlash). All solvents and chemicals were purchased from commercial suppliers and used without further purification. All reactions were capped from the atmosphere unless otherwise stated. NMR was recorded on a Varian Mercury Plus NMR (300 MHz) using $CDCl_3$ (7.27 ppm standard) or DMSO-d_6 (2.50 ppm standard). Data for ^{1}H NMR were recorded as follows: δ chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; dd, doublet of doublets; dt, doublet of triplets; q, quartet; dq, doublet of quartets; m, multiplet; br, broad; etc.), coupling constant (Hz), integration. Mass spectra were obtained on a LCQ Fleet Ion Trap LCMS, a micromass ZMD 1000 or PE Sciex API 150EX LCMS using electrospray ionization (ESI), or APCI mode. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. HRMS analyses were performed by the Mass Spectrometry Service Laboratory, University of Minnesota Department of Chemistry, Minneapolis, MN on a Bruker BioTOF II HRMS using ESI mode. HPLC analysis was performed on a reverse phase Agilent Zorbax SB-Phenyl column (5 μm, 2.1 x150 mm), using a binary gradient of 95:5→5:95 solvent A (95/5 H_2O/ACN + 0.1% formic acid): solvent B (5/95 H_2O/ACN + 0.1% formic acid) for 12 minutes, at a flow rate of 0.40 mL/min. Eluted peaks were monitored at 254 nm with a Shimadzu SPD-10AVP UV-Vis detector. All final compounds were confirmed to be of ≥95% purity by the HPLC method described above. Purity of final compounds was confirmed by elemental analysis and was within ±0.4% of the theoretical value. Molecular weights of selected compounds were also confirmed by HRMS, as indicated in the

experimental section. Based on ^{1}H NMR, HPLC, combustion analysis data and HRMS, all final compounds were ≥95% pure.

SYNTHESIS

General Procedure A: Reductive Amination. The aniline or amine substrate (1.00-2.00 equiv) and carbonyl compound (1.00-1.50 equiv) were charged into a round bottom flask. 1,2-DCE (0.030-0.25M) was added, and the mixture was stirred until both components dissolved. NOTE: If amine was a salt, *i*Pr$_2$NEt (2.30 equiv) was added at this stage to freebase. To this solution was added glacial AcOH (1.00-2.30 equiv) at ambient temperature, and the solution was stirred for 60 minutes. At this stage, sodium triacetoxyborohydride (STAB) (1.50-3.00 equiv) was added. The reaction was allowed to stir at room temperature and monitored by TLC (EtOAc:Hexanes). After 1-2 days, the reaction was ≥90% complete by TLC analysis. The reaction was quenched with saturated $NaHCO_3$ (aq.) and stirred until the reaction mixture was basic and bubbling had ceased. The biphasic layer was separated, and the organic layer was washed 2x with H_2O, brine, dried with $MgSO_4$, filtered and concentrated in vacuo to provide a crude product that was purified via flash chromatography to provide the desired product.

General Procedure B: Boc Removal (Step 1) and Reductive Amination with 4-isopropylcylohexanone (Step 2). *Step 1.* A solution of N-Boc intermediate (1.00 equiv) in CH_2Cl_2 (0.25-0.30M) was cooled to 0˚C, and then TFA (6-30 equiv) was added over several minutes. Upon completion of addition, the ice-bath was removed and the reaction was allowed to warm to room temperature and monitored by TLC (EtOAc:Hexanes). After 2 hours, the reaction was complete. The reaction was concentrated in vacuo, followed by the addition of EtOAc, which was consequently removed in vacuo to ensure the majority of excess TFA was removed. The resulting oily residue was dissolved in EtOAc and was stirred as saturated $NaHCO_3$ (aq.)

was added until the aqueous layer remained basic. The layers were separated, and the aqueous layer was extracted with EtOAc until UV activity in the aqueous layer was minimal (3-8x). The EtOAc layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide the piperidine intermediate.

Step 2. The piperidine intermediate from the previous step (1.00 equiv) and 4-*i*Pr-cyclohexanone (1.00-1.50 equiv) were dissolved in 1,2-DCE (0.070M). To the reaction was added glacial AcOH (1.00-2.30 equiv), and the reaction was stirred for 30 minutes. After 30 minutes, STAB (1.50-2.30 equiv) was added. An Ar balloon was fitted on top of the reaction, and the reaction was monitored by TLC (MeOH:CH_2Cl_2:NH_4OH (aq.)). After 2-3 days, the reaction was ≥95% complete. Saturated $NaHCO_3$ (aq.) was added until the aqueous layer remained basic. At this stage, the layers were separated, and the aqueous layer was extracted 2x with CH_2Cl_2. The organic layers were combined, and washed 2x with H_2O, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a crude residue that was purified via flash chromatography using EtOAc:Hexanes:NH_4OH (aq.).

General Procedure C: Formation of thiourea from amine. The amine (1.00 equiv) was dissolved in CH_2Cl_2 (0.10 M) at room temperature, and then the reaction was placed in an ice-bath and cooled to 0˚C. Benzoyl isothiocyanate (1.05-1.10 equiv) was added, and after several minutes of stirring at 0˚C, the reaction was allowed to warm to room temperature and monitored by TLC (EtOAc:Hexanes). After 1-3 hours, the reaction was complete by TLC. The reaction was concentrated in vacuo, the remaining residue was dissolved in MeOH (0.050 M), and to this was added K_2CO_3 (7.00 equiv). The reaction was stirred at room temperature and monitored by TLC (EtOAc:Hexanes). Once the reaction was complete, it was filtered over a pad of Celite, rinsed with MeOH, and then concentrated in vacuo to provide a crude material that was

purified via flash chromatography using EtOAc:Hexanes:NH_4OH (aq.) to provide the desired thiourea product.

tert-butyl 4-(indolin-1-yl)piperidine-1-carboxylate (**3**). See General Procedure A: Indoline **1** (10.0 g, 83.9 mmol, 1.00 equiv), N-Boc piperidone **2** (17.6 g, 88.1 mmol, 1.05 equiv), AcOH, 4.80 mL, 83.9 mmol, 1.00 equiv), STAB (26.7 g, 12.6 mmol, 1.50 equiv), DCE (352 mL, 0.25M). The crude oil was purified via flash chromatography using 10:90 EtOAc:Hexanes to provide indoline **3** as a white solid (24.3 g, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (t, *J* = 6.0 Hz, 2H), 6.03 (t, *J* = 6.0 Hz, 1H), 6.43 (d, *J* = 6.0 Hz, 1H), 4.25 (m, 2H), 3.52 (m, 1H), 3.35 (t, *J* = 6.3 Hz, 2H), 2.79 (m, 2H), 1.80 (d, *J* = 9.3 Hz, 2H), 1.60 (m, 4H), 1.49 (s, 9H); MS (APCI) *m/z*: 303.06 [M+H]+.

cis-1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)indoline (**4**). See General Procedure B: Step 1 (Boc Removal): Indoline **3** (24.4 g, 80.5 mmol, 1.00 equiv), TFA (38.0 mL, 496 mmol, 6.20 equiv), CH_2Cl_2 (300 mL, 0.27M). NOTE: Combined EtOAc layers were dried immediately with $MgSO_4$, and were not washed with water or brine, as the product is water-soluble. The product was obtained as a grey solid (13.6 g, 84% yield); Step 2 (Reductive Amination): N-H piperidine from the previous step (13.6 g, 67.2 mmol, 1.00 equiv), *i*Pr-cyclohexanone (9.40 g, 67.2 mmol, 1.00 equiv), AcOH (3.85 mL, 67.2 mmol, 1.00 equiv), STAB (21.3 g, 101 mmol, 1.50 equiv), DCE (960 mL, 0.070M). Purified via flash chromatography using 10:90:1.5 EtOAc:Hexanes:NH_4OH (aq.) to provide compound **4** as a light-gold oil (33% yield). R_f = 0.25 (20:80:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, pAA, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (t, *J* = 5.7 Hz, 2H), 6.60 (t, *J* = 5.7 Hz, 1H), 6.41 (d, *J* = 5.7 Hz, 1H), 3.37 (m, 3H), 3.10 (d, *J* = 8.7 Hz, 2H), 2.94 (t, *J* = 6.3 Hz, 2H), 2.27 (m, 1H), 2.14 (t, *J* = 8.7 Hz, 2H), 1.82-1.54 (m, 11H), 1.38 (m, 2H), 1.13 (m, 1H), 0.88 (d, *J* = 5.1 Hz, 6H); MS(ESI) *m/z*: 327.4 [M+H]+. The HCl salt

of amine **4** was formed by dissolving the amine in CH_2Cl_2 (0.10M) at room temperature, and cooling the reaction in a 0˚C ice-bath. To this solution was added HCl (2.0M in Et_2O, 1.50 equiv), and the resulting mixture was allowed to warm to room temperature and stir for 10 minutes. At this time, the reaction was concentrated in vacuo, Et_2O was added, and the mixture was concentrated in vacuo to provide a brown solid (repeat 3x total). This solid was then triturated w/ ca. 5-10% MeOH:Et_2O to provide the HCl salt of compound **4** as a cream solid. Anal. Calcd for $C_{22}H_{34}N_2 \cdot 1.00\ HCl \cdot 0.10\ H_2O$: C, 72.44; H, 9.73; N, 7.68; found: C, 72.39; H, 9.79; N, 7.34.

cis-1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indole (**5**). Indoline **4** (4.63 g, 14.2 mmol, 1.00 equiv) was dissolved in 180 mL of CH_2Cl_2. To this solution was added 4ÅMS (56.8 g, 4g/mmol of indoline), followed by MnO_2 (12.3 g, 142 mmol, 10.0 equiv) and another 20 mL of CH_2Cl_2. A balloon filled with argon was fitted on the reaction, and the thick suspension was stirred and monitored by TLC (20:80:3 drops EtOAc:Hexanes:NH_4OH (aq.)). After 16 hours, the reaction was complete. The mixture was filtered over a large pad of Celite and the remaining solid was washed 5x with CH_2Cl_2. The filtrate was concentrated in vacuo to provide a crude oil. This material was dissolved in EtOAc, and 10% HCl (aq.) was added with vigorous stirring, which resulted in a white precipitate. The white solid was filtered, and washed 3x with EtOAc, and was then air-dried over 1 hour. The white solid was then suspended in EtOAc, 70% $NaHCO_3$ (aq.) was added, and the mixture stirred until >90% of the solid had dissolved (usually overnight). The EtOAc layer was separated, washed with H_2O, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a thick oil that was purified via flash chromatography using 10:90:1.5 EtOAc:Hexanes:NH_4OH (aq.) to provide indole **5** as an off-white solid (3.65 g, 79% yield). R_f = 0.25 (10:90:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, pAA, I_2); ^1H NMR (300

MHz, $CDCl_3$) δ 7.64 (d, *J* = 6.0 Hz, 1H), 7.39 (d, *J* = 6.0 Hz, 1H), 7.26 (m, 1H), 7.20 (t, *J* = 6.0 Hz, 1H), 7.11 (t, *J* = 6.0 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 4.23 (m, 1H), 3.20 (d, *J* = 9.0 Hz, 2H), 2.30 (m, 3H), 2.08 (m, 4H), 1.51-1.78 (m, 7H), 1.40 (m, 2H), 1.17 (m, 1H), 0.90 (d, *J* = 4.8 Hz, 6H); MS(ESI) *m/z*: 325.4 [M+H]+. Anal. Calcd for $C_{22}H_{32}N_2$: C, 81.43; H, 9.94; N, 8.63; found: C, 81.54; H, 9.87; N, 8.45.

cis-1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indole-3-carboxamide (**6**). Indole **5** (60.0 mg, 0.185 mmol, 1.00 equiv) was dissolved in 1.00 mL of MeCN (0.19M) with the aid of heat. The solution was allowed to cool to room temperature, and then it was placed in an ice bath for several minutes. Chlorosulfonylisocyanate (20.1 μL, 0.231 mmol, 1.25 equiv) was then added, turning the reaction light-yellow. The reaction was stirred at 0˚C and monitored by TLC (20:80 EtOAc:Hexanes). After 30 minutes, TLC showed full consumption of indole **5**. Water was added to the reaction, temporarily resulting in a white precipitate. The ice-bath was removed, and the mixture was allowed to warm to room temperature and stir overnight, resulting in a light-yellow solution. The reaction was then quenched with saturated $NaHCO_3$ (aq.), and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted 2x with EtOAc. The EtOAc layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide the crude product. Amide **6** was purified via flash chromatography using 2:96:1 MeOH:EtOAc:NH_4OH (aq.) to provide amide **6** as a white solid (25 mg, 37% yield). R_f = 0.30 (5:95:3 drops MeOH:EtOAc:NH_4OH (aq.), UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (m, 1H), 7.90 (s, 1H), 7.46-7.43 (m, 1H), 7.30-7.27 (m, 2H), 5.78 (br s, 2H), 4.23 (m, 1H), 3.22 (d, *J* = 8.7 Hz, 2H), 2.37 (m, 2H), 2.28 (t, *J* = 8.7 Hz, 2H), 2.1 (m, 4H), 1.75-1.52 (m, 7H), 1.40 (m, 2H), 1.16 (m, 1H), 0.90 (d, *J* = 5.1 Hz, 6H); MS(ESI) *m/z*: 368.5

[M+H]+. Anal. Calcd for $C_{23}H_{33}N_3O \cdot 0.50$ MeOH: C, 73.59; H, 9.20; N, 10.96; found: C, 73.64; H, 8.82; N, 10.57.

cis-1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indole-3-carbaldehyde (**7**). To a stirred solution of 25.0 mL DMF at 0˚C was added $POCl_3$ (3.66 mL, 40.0 mmol, 4.00 equiv). The solution was stirred at 0˚C for 15 minutes. At this stage, indole **5** (3.10 g, 10.0 mmol, 1.00 equiv) was dissolved in 10 mL of DMF with the assistance of heat. The warm solution of indole **5** was then added to the reaction, and the reaction was rinsed with 5.00 mL of DMF. The addition of indole **5** leads to a red solution, and the reaction was allowed to stir for 15-20 minutes at 0˚C. TLC (50:50:3 drops EtOAc:Hexanes:NH_4OH (aq.)) showed the reaction was complete. The reaction was poured into a saturated $NaHCO_3$ (aq.) icebath, followed by the addition of CH_2Cl_2. The mixture was stirred vigorously for 30 minutes, upon which the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 until UV activity was minimal (5-6x). The organic layer was then washed 3x with H_2O, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a dark red oil, which was purified via flash chromatography using 50:50:1.5 EtOAc:Hexanes:NH_4OH (aq.) to provide aldehyde **7** as a light-yellow solid (2.15 g, 74% yield). R_f = 0.20 (50:50:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.0 (s, 1H), 8.33 (m, 1H), 7.89 (s, 1H), 7.43 (m, 1H), 7.33 (m, 2H), 4.29 (m, 1H), 3.28 (d, *J* = 7.8 Hz, 2H), 2.40 (m, 3H), 2.19 (m, 3H), 1.55-1.78 (m, 8H), 1.42 (m, 2H), 1.17 (m, 1H), 0.90 (d, *J* = 5.7 Hz, 6H); MS(ESI) *m/z*: 353.1 [M+H]+.

cis-1-(1-(4-isopropylcyclohexyl)piperidin-4-yl)-1H-indole-3-carbaldehyde oxime (**8**). Aldehyde **7** (2.15 g, 6.10 mmol, 1.00 equiv), $NH_2OH \cdot HCl$ (551 mg, 7.93 mmol, 1.30 equiv), and $NaOAc \cdot 3H_2O$ (1.08 g, 7.93 mmol, 1.30 equiv) were charged into a round bottom flask. Absolute EtOH (20.5 mL) and 10 mL of H_2O were added, and the reaction was fitted with a condenser and

an Ar balloon on top. The suspension was heated to reflux (ca. 110˚C oil bath) and monitored by TLC (40:60:3drops EtOAc:Hexanes:NH_4OH (aq.)). After 2 hours, the reaction was complete. The reaction was allowed to cool to room temperature upon which a white precipitate formed. The mixture was diluted with EtOAc and saturated $NaHCO_3$ (aq.), and stirred until the mixture became a biphasic solution. The layers were separated, and the organic layer was washed 2x with H_2O, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide oxime **8** as a white solid (2.13 g, 96% yield). The two isomers of the oxime are in ca. 3:2 ratio, and can be enriched via flash chromatography using 40:60:1→50:50:1 EtOAc:Hexanes:NH_4OH (aq.). R_f = 0.40 (top spot), 0.45 (bottom spot) (50:50:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, I_2); Isomer #1: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.8 (br s, 1H), 8.47 (s, 1H), 7.77 (m, 1H), 7.41 (d, *J* = 6.0 Hz, 1H), 7.28-7.20 (m, 3H), 4.31 (m, 1H), 3.30 (d, *J* = 8.7 Hz, 2H), 2.55 (m, 1H), 2.46 (t, *J* = 8.4 Hz, 2H), 2.22 (m, 3H), 1.86 (m, 2H), 1.78-1.57 (m, 6H), 1.43 (m, 2H), 1.18 (m, 1H), 0.91 (d, *J* = 5.1 Hz, 6H); Isomer #2: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.07 (d, *J* = 6.0 Hz, 1H), 7.48 (s, 1H), 7.40 (d, *J* = 6.0 Hz, 1H), 7.28 (m, 1H), 7.20 (t, *J* = 5.4 Hz, 1H), 4.23 (m, 1H), 3.21 (d, *J* = 8.7 Hz, 2H), 2.35 (m, 3H), 2.13 (m, 4H), 1.80-1.55 (m, 7H), 1.43 (m, 2H), 1.18 (m, 1H), 0.90 (d, *J* = 5.1 Hz, 6H); MS(ESI) *m/z*: 368.5 [M+H]+. Anal. Calcd for $C_{23}H_{33}N_3O$: C, 75.16; H, 9.05; N, 11.43; found: C, 75.27; H, 8.88; N, 11.30.

cis-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methanamine (**9**). In a 500 mL parr bottle was added oxime **8** (2.00 g, 5.44 mmol, 1.00 equiv), and it was then suspended in MeOH (46 mL). To this mixture was added Raney Nickel (2.00 g, 100 wt%, prewashed with MeOH) and concentrated NH_4OH (aq.) (23 mL). The reaction mixture was placed on the parr hydrogenator, and the parr vessel was pressurized with H_2 (g) at ca. 20 psi, then purged under vacuo. This was repeated a total of 3x. The vessel was the pressurized at 50

psi for 18 hours. The reaction mixture was filtered over Celite, and washed thoroughly with MeOH. The resulting filtrate was concentrated in vacuo, and the crude product was purified by flash chromatography using 8:92 MeOH:CH_2Cl_2 to provide amine **9** as a white solid (1.52 g, 78% yield). R_f = 0.30 (10:90:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.58 (d, *J* = 6.0 Hz, 1H), 7.44 (d, *J* = 6.3 Hz, 1H), 7.37 (s, 1H), 7.10 (t, *J* = 5.7 Hz, 1H), 6.98 (t, *J* = 5.7 Hz, 1H), 4.26 (m, 1H), 3.86 (s, 1H), 3.16 (s, 2H), 3.08 (d, *J* = 8.7 Hz, 3H), 2.27 (br s, 1H), 2.18 (t, *J* = 8.4 Hz, 2H), 1.91-1.86 (m, 4H), 1.71 (br s, 2H), 1.55-1.50 (m, 3H), 1.38 (dd, *J* = 18.3, 9.3 Hz, 4H), 1.10 (s, 1H), 0.86 (d, *J* = 5.1 Hz, 6H); MS(ESI) *m/z*: 354.5 [M+H]+. The bis HCl salt of amine **9** was formed by dissolving the amine in CH_2Cl_2 (0.10M) at room temperature, followed by the addition of HCl (2.0M in Et_2O, 2.20 equiv). The reaction was allowed to stir for 10 minutes. At this time, the reaction was concentrated in vacuo, Et_2O was added to the mixture, followed by concentrating the mixture in vacuo (repeat 3x total) to provide the HCl salt of amine **9**. Anal. Calcd for $C_{23}H_{35}N_3 \cdot 2.00$ HCl·1.50 H_2O: C, 60.92; H, 8.89; N, 9.27; found: C, 60.94; H, 8.76; N, 9.11.

cis-1-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)-N,N-dimethylmethanamine (**10**). In the reduction of of oxime **8** to amine **9**, a non-polar byproduct formed. Purification via flash chromatography using 3:97:1 MeOH:CH_2Cl_2:NH_4OH (aq.) provided the byproduct in low yield (10-15%) as a white solid, and the structure was elucidated as dimethyl amine **10**. R_f = 0.70 (10:90:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 6.0 Hz, 1H), 7.62 (d, *J* = 6.0 Hz, 1H), 7.37 (d, *J* = 6.0 Hz, 1H), 7.22 (d, *J* = 5.1 Hz, 1H), 7.16 (m, 1H), 5.61-5.43 (m, 6H), 4.87 (s, 1H), 4.65 (d, *J* = 3.9 Hz, 1H), 4.59 (d, *J* = 3.6 Hz, 1H), 4.19 (t, *J* = 5.4 Hz, 1H), 3.21 (d, *J* = 7.5 Hz, 2H), 2.38-2.30 (m, 3H), 2.18-1.98 (m, 6H), 1.71-1.60 (m, 5H), 1.43-1.37 (m, 2H), 1.14 (t, *J* = 3.3 Hz, 1H), 0.89 (d, *J* =

5.1 Hz, 6H); MS(ESI) *m/z*: 382.27 [M+H]+. HRMS Calcd for $C_{25}H_{39}N_3$ (M+H)+ 382.3144, found 382.2866.

cis-1-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)thiourea (11). See General Procedure C. Step 1. Amine **9** (200 mg, 0.565 mmol, 1.00 equiv), Benzoyl isothiocyanate (83.6 mg, 0.622 mmol, 1.10 equiv), CH_2Cl_2 (5.60 mL, 0.10 M). **Step 2.** K_2CO_3 (547 mg, 3.96 mmol, 7.00 equiv), MeOH (5.80 mL, 0.050 M). Obtained thiourea **11** as a light-yellow glue (70.0 mg, 58% yield). R_f = 0.50 (10:90:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (br s, 1H), 7.37 (d, J = 6Hz, 1H), 7.24 (s, 2H), 7.11 (t, J = 5.1 Hz, 1H), 6.58 (br s, 1H), 5.82 (s, 2H), 4.88 (br s, 1H), 4.45 (br s, 1H), 4.18 (s, 1H), 3.14 (d, J = 6.9 Hz, 2H), 2.33 (s, 1H), 2.22 (t, J = 9.3 Hz, 2H), 2.02-1.97 (m, 4H), 1.71-1.61 (m, 5H), 1.55 (m, 2H), 1.41 (m, 2H), 1.14 (m, 1H), 0.89 (d, J = 4.8 Hz, 6H); MS(ESI) m/z: 413.11 [M+H]+. The bis HCl salt of urea **11** was formed by dissolving the amine in CH_2Cl_2 (0.10M) at room temperature, followed by the addition of HCl (2.0M in Et_2O, 2.20 equiv). The reaction was allowed to stir for 10 minutes. At this time, the reaction was concentrated in vacuo, Et_2O was added to the mixture, followed by concentrating the mixture in vacuo (repeat 3x total) to provide the HCl salt of urea **11**. Anal. Calcd for $C_{24}H_{36}N_4S \cdot 2.00\ HCl \cdot 1.00\ CH_2Cl_2$: C, 55.76; H, 7.48; N, 10.40; found: C, 55.66; H, 7.52; N, 10.74.

cis-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methanol (12). Aldehyde **7** (1.50 g, 4.26 mmol, 1.00 equiv) was taken up in absolute EtOH (50 mL, 0.085M), and sodium borohydride (806 mg, 21.3 mmol, 5.00 equiv) was then added in several portions at room temperature. After bubbling had subsided, the reaction was stirred for an addition 30 minutes and then concentrated in vacuo. The resulting white residue was suspended in a 5% NaOH (aq.) solution (20 mL) and extracted 3x with EtOAc. The EtOAc layer was washed with

water, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a crude product that was purified via a 2 inch long filter column using 8:92 EtOAc:Hexanes to provide alcohol **12** as a pale white solid (1.4 g, 86% yield). R_f = 0.30 (10:90 EA:Hexanes, UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 5.7 Hz, 1H), 7.37 (d, *J* = 6.0 Hz, 1H), 7.26 (s, 1H), 7.22 (d, *J* = 6.0 Hz, 1H), 7.13 (t, *J* = 5.4 Hz, 1H), 4.87 (s, 2H), 4.20 (m, 1H), 3.15 (d, *J* = 8.7 Hz, 2H), 2.33 (br s, 1H), 2.23 (t, *J* = 8.4 Hz, 2H), 1.98 (dd, *J* = 18.3, 9.0 Hz, 4H), 1.73-1.69 (m, 4H), 1.61 (t, *J* = 4.8 Hz, 2H), 1.57 (m, 1H), 1.41 (m, 2H), 1.26 (br s, 1H), 1.02 (br s, 1H), 0.86 (dd, *J* = 4.8, 6.9 Hz, 6H); MS(ESI) *m/z*: 355.22 [M+H]+. Anal. Calcd for $C_{23}H_{34}N_2O \cdot 0.20\ CH_2Cl_2$: C, 75.00; H, 9.33; N, 7.54; found: C, 74.62; H, 9.62; N, 7.34.

Synthesis of alkynes 15 and 16.

tert-butyl (4-(triethylsilyl)but-3-yn-1-yl)carbamate (**15**). Alkyne **15** was obtained as a light-yellow oil using the same 4-step synthesis that provided alkyne **16**. Step 1: 99% yield. Step 2: 94% yield. Step 3: 70% yield. Step 4: 78% yield. R_f = 0.10 (5:95 EtOAc:Hexanes, I_2 and pAA); 1H NMR (300 MHz, $CDCl_3$) δ 4.80 (br, 1H), 3.28 (q, *J* = 6.6 Hz, 2H), 2.45 (t, *J* = 6.6 Hz, 2H), 1.45 (s, 9H), 0.99 (t, *J* = 7.8 Hz, 9H), 0.58 (q, *J* = 7.8 Hz, 6H).

tert-butyl (5-(trimethylsilyl)pent-4-yn-1-yl)carbamate (**16**). To a solution of 5-(trimethylsilyl)pent-4-yn-1-ol (**14**) (2.55 g, 16.3 mmol, 1.00 equiv) (GFS Chemicals) in 65.2 mL of CH_2Cl_2 (0.25M) at room temperature, was added triethylamine (2.95 mL, 21.2 mmol, 1.30 equiv) and DMAP (99.6 mg, 0.815 mmol, 0.050 equiv). The solution was stirred for several minutes, and then TsCl (3.36 g, 17.6 mmol, 1.08 equiv) was added at room temperature. The reaction was fitted with an Ar balloon and allowed to stir for 16 hours. At this stage, TLC (10:90 EtOAc:Hexanes) showed the reaction was complete. The reaction was concentrated in vacuo to half of the initial reaction volume, then diluted with EtOAc and water, and the biphasic solution

stirred for five minutes. The layers were then separated, and the aqueous layer was extracted 2x with EtOAc. The EtOAc layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide the tosylate intermediate used directly in the following step (4.70 g, 93% yield).

The tosylate intermediate (4.70 g, 15.1 mmol, 1.00 equiv) and phthalimide (2.78 g, 18.9 mmol, 1.25 equiv) were dissolved in 60.0 mL of DMF (0.25M) at room temperature. K_2CO_3 (6.27 g, 45.4 mmol, 3.00 equiv) was added, the reaction was fitted with an Ar balloon, and the reaction was heated to 50˚C for 6 hours. At this time, TLC (10:90 EtOAc:Hexanes) shows the reaction is complete. The reaction was allowed to cool to room temperature, and then it was diluted with EtOAc and water. The layers were separated, and the aqueous layer was extracted 2x with EtOAc. The EtOAc layers were combined, washed 3x with water, 1x with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide semi-crude material. This material was purified via flash chromatography using 8:92 EtOAc:Hexanes to provide the phthalimide intermediate as a white solid (2.89 g, 67% yield). This material was used directly in the following step.

The phthalimide intermediate (2.20 g, 7.71 mmol, 1.00 equiv) from the previous step was dissolved in 77.0 mL of MeOH (0.10M) at room temperature, and then hydrazine (0.966 mL, 30.8 mmol, 4.00 equiv) was added, and the reaction was allowed to stir at room temperature overnight. At this stage, a large amount of a white precipitate had formed. The white precipitate was filtered, washed thoroughly with MeOH, and the resulting filtrate was concentrated in vacuo to provide a solid-liquid mixture. This mixture was diluted with EtOAc, stirred several minutes, and the resulting solids were filtered, and washed thoroughly with EtOAc. The filtrate (EtOAc),

was then washed with water, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide the desired amine (863 mg, 72% yield), which is used directly in the following step.

The amine intermediate (863 mg, 5.56 mmol, 1.00 equiv) and DMAP (34.0 mg, 0.278 mmol, 0.050 equiv) were dissolved in 37 mL of THF (0.15M) at room temperature. To this solution was added Boc anhydride (1.33 g, 6.11 mmol, 1.10 equiv), and the reaction was allowed to stir overnight. At this stage, TLC (2:98:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.)) showed the reaction was complete. The reaction was concentrated in vacuo, and the crude mixture was purified via flash chromatography using 5:95 EtOAc:Hexanes to provide alkyne **16** as a clear oil (1.25 g, 88% yield). R_f = 0.10 (5:95 EtOAc:Hexanes, I_2 and pAA); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.80 (br, 1H), 3.23 (m, 2H), 2.29 (t, J = 5.4 Hz, 2H), 1.71 (m, 2H), 1.49 (s, 9H), 0.16 (s, 9H).

cis-N-(2-iodophenyl)-1-(-4-isopropylcyclohexyl)piperidin-4-amine (18).

i. See General Procedure A. 2-iodoaniline (**17**) (15.0 g, 63.3 mmol, 1.00 equiv), N-Boc-piperidone (**2**) (18.5 g, 95.0 mmol, 1.50 equiv), glacial AcOH (8.40 mL, 146 mmol, 2.30 equiv), STAB (30.9 g, 146 mmol, 2.30 equiv), DCE (250 mL, 0.25M). Purified via flash chromatography using 5:95 EtOAc:Hexanes to provide the secondary aniline intermediate as a white solid (19.1 g, 75% yield).

ii. See General Procedure B. **Step 1:** Aniline intermediate (43.5 g, 0.108 mol, 1.00 equiv), TFA (200 mL, 2.61 mol, 24.0 equiv), CH_2Cl_2 (300 mL, 0.36M). Obtained the N-H piperidine intermediate as a light-tan solid (42.0 g, 128% yield, due to NaTFA), and was used directly in the next step. **Step 2:** N-H piperidine (assume 0.108 mol, 1.00 equiv), 4-*i*Pr-cyclohexanone (22.7 g, 0.162 mol, 1.50 equiv), glacial AcOH (14.2 mL, 0.248 mol, 2.30 equiv), STAB (52.6 g, 0.248 mol, 2.30 equiv), DCE (1.54 L, 0.070M). Compound **18** was separated from its anti-diastereomer via flash chromatography using 6:94:1.5→9:91:1.5

EtOAc:Hexanes:NH_4OH (aq.) to provide a golden oil (The syn diastereomer has a higher R_f value compared to the anti diastereomer). Although the product is pure when viewed under UV light (short wave), the product contains non-UV active impurities (viewable with pAA) related to 4-*i*Pr-cyclohexanone. Thus, to remove these impurities the golden oil was dissolved in EtOAc (ca. 300 mL) and transferred to an erlenmeyer flask. At this stage, 10% HCl (aq.) (ca. 200 mL) was added. Upon addition of the 10% HCl (aq.), a white precipitate formed, and the suspension was stirred for 10 minutes. The white precipitate was then filtered, washed 2x with EtOAc, and then air-dried over 1 hour. The white precipitate was then placed in an erlenmeyer flask, suspended in EtOAc, and 70% $NaHCO_3$ (aq.) was added until basic. The mixture was then stirred overnight. At this stage, the mixture is now a clear biphasic solution (add water and stir if significant amount of white precipitate remain). The layers were separated, and the EtOAc layer was washed with water, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide iodoaniline **18** as a light-gold oil (24.0 g, 39% yield over 3 steps). R_f = 0.30 (10:90:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (dd, *J* = 5.7, 0.9, 1H), 7.18 (t, *J* = 6.0, 1H), 6.58 (d, *J* = 6.0, 1H), 6.41 (dt, *J* = 5.7, 0.9, 1H), 4.12 (d, *J* = 5.7 Hz, 1H), 3.36 (m, 1H), 2.93 (m, 2H), 2.25 (m, 3H), 2.15 (d, *J* = 8.4 Hz, 2H), 1.47-1.74 (m, 8H), 1.38 (m, 2H), 1.13 (m, 1H), 0.89 (d, *J* = 4.8 Hz, 6H); MS(ESI) *m/z*: 427 [M+H]+.

cis-tert-butyl (2-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-2-(triethylsilyl)-1H-indol-3-yl)ethyl)carbamate (**19**). Iodoaniline **18** (1.00 g, 2.35 mmol, 1.00 equiv) and alkyne **15** (797 mg, 2.81 mmol, 1.20 equiv) were charged into a round-bottom flask. LiCl (99.4 mg, 2.35 mmol, 1.00 equiv) was added, and the mixture was diluted with 33.5 mL of DMF (0.070M). K_2CO_3 (973 mg, 7.04 mmol, 3.00 equiv) was added to the reaction, followed by a catalytic amount of $Pd(OAc)_2$ (26.3 mg, 0.117 mmol, 0.050 equiv). The reaction was fitted with an Ar

balloon, purged 3x under vacuum and backfilled with Ar, and heated in a 102˚C oil bath. The reaction was monitored by TLC, and was complete at ca. 2.5 hours. NOTE: Pd0 (black) was observed ca. 2 hours into reaction. Once the reaction was complete, the mixture was allowed to cool to room temperature and was diluted with EtOAc and water. The mixture was stirred for several minutes, and then filtered. The filtrate layers were separated, and the aqueous layer was extracted 1x with EtOAc. The EtOAc layers were combined and washed 2x with water, 1x with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a crude product that was purified via flash chromatography using 7:93:1→10:90:1 EtOAc:Hexanes:NH_4OH (aq.) to provide indole **19** as a white foam (1.04 g, 76% yield). R_f = 0.25 (10:90:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, I_2); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.69 (d, *J* = 6.3 Hz, 1H), 7.62 (d, *J* = 5.7 Hz, 1H), 7.16 (t, *J* = 5.7 Hz, 1H), 7.06 (t, *J* = 5.7 Hz, 1H), 4.56 (br, 1H), 4.25 (m, 1H), 3.40 (m, 2H), 3.21 (d, J = 8.4 Hz, 2H), 3.02 (t, J = 5.1 Hz, 2H), 2.71 (dq, J = 9.0, 2.1 Hz, 2H), 2.35 (m, 1H), 2.17 (t, J = 9.0 Hz, 2H), 1.38–1.75 (m, 20H), 1.16 (m, 1H), 0.90–1.03 (m, 21H); MS(ESI) m/z: 582.8 [M+H]+.

cis-tert-butyl (3-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-2-(trimethylsilyl)-1H-indol-3-yl)propyl)carbamate (20). Indole **20** was synthesized in a similar manner as indole **19**. Iodoaniline **20** (720 mg, 1.69 mmol, 1.00 equiv), alkyne **16** (475 mg, 1.86 mmol, 1.10 equiv), LiCl (71.6 mg, 1.69 mmol, 1.00 equiv), K_2CO_3 (700 mg, 5.07 mmol, 3.00 equiv), $Pd(OAc)_2$ (38.0 mg, 0.169 mmol, 0.100 equiv), and DMF (24.0 mL, 0.070M). Reaction Time of ca. 6 hours. Purified via flash chromatography using 10:90:1 EtOAc:Hexanes:NH_4OH (aq.) to provide indole **20** in ca. 90% purity as light-beige solid (88% yield). Trituration using MeOH provided pure indole **20** as a white solid (585 mg, 65% yield). R_f = 0.10 (10:90:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, I_2); MS(ESI) m/z: 554.8 [M+H]+.

cis-2-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)ethan-1-amine (21). AcCl (806 μL, 11.3 mmol, 6.00 equiv) was added to 19.0 mL of MeOH (0.10M) at 0˚C, and the mixture was stirred for several minutes at 0˚C. At this stage, indole intermediate **19** (1.10 g, 1.89 mmol, 1.00 equiv) was added, resulting in a slurry. The ice-bath was then removed, and the reaction was allowed to warm to room temperature and stir overnight. Once TLC confirmed the reaction was complete, EtOAc (ca. 5x the amount of MeOH) was added and the reaction was allowed to stir for 60 minutes, during which a white precipitate formed. The white precipitate was then filtered and washed 3x with EtOAc. The white solid was then collected, and dried in vacuo to provide the bis HCl salt of indole **21** as a white solid (665 mg, 80% yield). R_f = 0.10 (10:90:3 drops iPrOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, J = 6.0 Hz, 1H), 7.36 (d, J = 6.3 Hz, 1H), 7.21 (t, J = 6.0 Hz, 1H), 7.11 (m, 2H), 4.18 (m, 1H), 3.19 (d, J = 8.7 Hz, 2H), 3.03 (t, J = 4.8 Hz, 2H), 2.93 (t, J = 4.8 Hz, 2H), 2.35 (m, 1H), 2.26 (dt, J = 8.4, 1.8 Hz, 2H), 2.07 (br, 6H), 1.76-1.53 (m, 7H), 1.42 (m, 2H), 1.14 (m, 1H), 0.90 (d, J = 5.1 Hz, 6H); MS(ESI) m/z: 368.6 [M+H]+. Anal. Calcd for $C_{24}H_{37}N_3 \cdot 2.00\ HCl \cdot 0.50\ H_2O$: C, 64.12; H, 8.97; N, 9.35; found: C, 64.23; H, 8.73; N, 9.32.

cis-3-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)propan-1-amine (22). Compound **22** was synthesized in a similar manner as indole **21**. Indole intermediate **20** (582 mg, 1.09 mmol, 1.00 equiv), AcCl (466 μL, 6.55 mmol, 6.00 equiv), MeOH (7.3 mL, 0.15M). Obtained the bis HCl salt of indole **22** as a white solid (388 mg, 78% yield). R_f = 0.10 (5:95:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2); ^{1}H NMR (CD_3OD, 300 MHz) δ 7.58 (d, J = 6.0 Hz, 1H), 7.50 (d, J = 6.3 Hz, 1H), 7.25 (s, 1H), 7.19 (t, J = 6.0 Hz, 1H), 7.07 (t, J = 6.0 Hz, 1H), 4.72 (m, 1H), 3.74 (d, J = 9.6 Hz, 2H), 3.40-3.30 (m, 5H), 2.98 (t, J = 5.7 Hz, 2H), 2.88 (t, J = 5.7 Hz, 2H), 2.50 (dq, J = 9.6, 2.4 Hz, 2H), 2.28 (d, J = 9.9 Hz, 2H), 2.10-1.95 (m, 6H), 1.80

(m, 3H), 1.57 (t, J = 9.9 Hz, 2H), 1.28 (m, 1H), 0.97 (d, J = 5.1 Hz, 6H); MS(ESI) m/z: 382.6 [M+H]+. Anal. Calcd for $C_{25}H_{39}N_3 \cdot 2.00\ HCl \cdot 0.50\ H_2O$: C, 64.78; H, 9.13; N, 9.07; found: C, 64.93; H, 9.11; N, 9.04.

cis-1-(2-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)ethyl)thiourea (23). See General Procedure C. Step 1. Amine **21** (35.0 mg, 95.2 μmol, 1.00 equiv), Benzoyl isothiocyanate (13.5 μL, 100 μmol, 1.05 equiv), CH_2Cl_2 (950 μL, 0.10 M). **Step 2.** K_2CO_3 (92.0 mg, 666 μmol, 7.00 equiv), MeOH (1.90 mL, 0.050 M). Obtained thiourea **23** as a light-yellow glue (20.2 mg, 50% yield). R_f = 0.15 (60:40:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, I_2); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.57 (br s, 1H), 7.37 (d, J = 6.3 Hz, 1H), 7.23 (t, J = 5.4 Hz, 1H), 7.12 (m, 2H), 6.27 (br s, 1H), 5.84 (br s, 2H), 4.20 (m, 1H), 3.22 (d, J = 8.4 Hz, 2H), 3.06 (t, J = 4.8 Hz, 2H), 2.42-2.07 (m, 7H), 1.78-1.56 (m, 7H), 1.41 (m, 2H), 1.27 (m, 2H), 1.16 (m, 1H), 0.91 (d, J = 5.1 Hz, 6H); MS(ESI) m/z: 427.7 [M+H]+. HRMS (ESI) calcd for C25H39N4S (M+H), 427.2895; found, 427.2896.

cis-1-(3-(1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)propyl)thiourea (24). See General Procedure C. Step 1. Amine **22** (20.0 mg, 52.4 μmol, 1.00 equiv), Benzoyl isothiocyanate (7.40 μL, 55.0 μmol, 1.05 equiv), CH_2Cl_2 (525 μL, 0.10 M). **Step 2.** K_2CO_3 (50.0 mg, 0.367 mmol, 7.00 equiv), MeOH (1.05 mL, 0.050 M). Obtained thiourea **24** as a clear oil (10.8 mg, 47% yield). R_f = 0.20 (50:50:3 drops EtOAc:Hexanes:NH_4OH (aq.), UV, pAA, I_2); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.56 (d, J = 6.0 Hz, 1H), 7.36 (d, J = 6.0 Hz, 1H), 7.21 (t, J = 5.7 Hz, 1H), 7.10 (m, 2H), 6.26 (br s, 1H), 5.66 (br s, 2H), 4.18 (m, 1H), 3.18 (m, 3H), 2.85 (t, J = 5.4 Hz, 2H), 2.40-2.25 (m, 3H), 2.12-1.90 (m, 6H), 1.77-1.54 (m, 6H), 1.42 (m, 2H), 1.26 (m, 2H), 1.15 (m, 1H), 0.90 (d, J = 4.8 Hz, 6H); MS(ESI) m/z: 427.7 [M+H]+. Anal. Calcd for $C_{26}H_{40}N_4S \cdot 0.50\ H_2O$: C, 69.44; H, 9.19; N, 12.46; found: C, 69.43; H, 9.13; N, 12.07.

cis-1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-3-(pyrrolidin-1-ylmethyl)-1H-indole (**25**). See General Procedure A. Conditions: aldehyde **7** (150 mg, 0.426 mmol, 1.00 equiv.), pyrrolidine (57.0 μL, 0.682 mmol, 1.60 equiv), glacial AcOH (56.1 μL, 0.980 mmol, 2.30 equiv), STAB (208 mg, 0.980 mmol, 2.30 equiv), DCE (4.30 mL, 0.10M). The crude product was purified via flash chromatography using 0:100:1→2:98:1 MeOH:EtOAc:NH_4OH (aq.) to provide indole **25** as an oil (93 mg, 53% yield).). R_f = 0.10 (100:3 drops EtOAc:NH_4OH (aq.), UV, I_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.24 (s, 1H), 7.20 (t, *J* = 7.5 Hz, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 4.19 (m, 1H), 3.84 (s, 2H), 3.18 (d, *J* = 11.7 Hz, 2H), 2.60 (m, 4H), 2.35-2.19 (m, 3H), 2.12-1.97 (m, 4H), 1.81-1.48 (m, 11H), 1.40 (m, 2H), 1.16 (m, 1H), 0.91 (d, *J* = 6.6 Hz, 6H); MS(ESI) *m/z*: 408.6 [M+H]+. The HCl salt of amine **25** was formed by dissolving the amine in CH_2Cl_2 (0.10M) at room temperature, and cooling the reaction in a 0˚C ice-bath. To this solution was added HCl (2.0M in Et_2O, 3.00 equiv), and the reaction was allowed to warm to room temperature and stir for 30 minutes. At this time, the reaction was concentrated in vacuo, Et_2O was added, and the mixture was concentrated in vacuo (repeat 3x total) to provide an off-white solid. Anal. Calcd for $C_{27}H_{41}N_3 \cdot 2.00\ HCl \cdot 1.50\ H_2O$: C, 63.89; H, 9.14; N, 8.28; found: C, 63.92; H, 8.92; N, 8.26.

cis-1-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)piperidin-4-amine (**27**). *Step 1.* See General Procedure A. Conditions: aldehyde **7** (100 mg, 0.284 mmol, 1.00 equiv.), 4-(N-Boc-amino)piperidine (56.0 mg, 0.284 mmol, 1.00 equiv), Note: glacial AcOH not used, STAB (180 mg, 0.851 mmol, 3.00 equiv), DCE (10.0 mL, 0.03M). The crude Boc-protected amine (**26**), (0.153 g, 98% yield) was taken directly into the Boc removal step.

Step 2. Boc-protected amine **26** (85.0 mg, 0.160 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (5.0 mL, 0.030M), followed by the addition of excess TFA (4.00 mL). The reaction

mixture was stirred for 10 minutes, and then the solvent was evaporated to provide a yellow oily product. This was triturated with Et_2O to provide the TFA salt of indole **27** as a solid (97.5 mg, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 4.2, 2.4 Hz, 2H), 7.53 (dd, *J* = 4.2, 2.4 Hz, 2H), 7.40 (br s, 1H), 4.22 (m, 3H), 3.48 (dd, *J* = 10.5, 5.1 Hz, 4H), 2.08 (s, 14H), 1.69 (m, 2H), 1.43 (m, 3H), 1.35-1.29 (m, 4H), 1.20 (t, *J* = 5.1 Hz, 3H), 0.94-0.88 (m, 6H); MS(ESI) *m/z*: 437.50 [M+H]+. Anal. Calcd for $C_{28}H_{44}N_4$·3.00 TFA·1.00 Et_2O·1.00 CH_2Cl_2: C, 50.25; H, 6.22; N, 5.77; found: C, 50.26; H, 6.01; N, 5.60.

cis-1-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)piperidin-4-ol (**28**). See General Procedure A. Conditions: aldehyde **7** (205 mg, 0.630 mmol, 1.00 equiv.), piperin-4-ol·HCl (173 mg, 1.26 mmol, 2.00 equiv), *i*Pr_2NEt (253 μL, 1.45 mmol, 2.30 equiv), glacial AcOH (83.0 μL, 1.45 mmol, 2.30 equiv), STAB (307 mg, 1.45 mmol, 2.30 equiv), DCE (6.30 mL, 0.10M). The crude product was purified via flash chromatography using 3:97:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to afford indole **28** as a light-yellow foam (140 mg, 51% yield). R_f = 0.15 (5:95:1 MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.24-7.17 (m, 2H), 7.11 (t, *J* = 6.9 Hz, 1H), 4.42 (m, 1H), 3.75 (m, 3H), 3.19 (d, *J* = 12.0 Hz, 2H), 2.85 (m, 2H), 2.38-2.20 (m, 4H), 2.18-1.85 (m, 4H), 1.78-1.35 (m, 14H), 1.17 (m, 1H), 0.91 (d, *J* = 6.6 Hz, 6H); MS(ESI) *m/z*: 438.7 [M+H]+. The bis tartrate salt of amine **28** was formed by dissolving the amine in MeOH (0.10M) at room temperature. To the reaction was added L-(+)-tartaric acid (2.00 equiv) in a MeOH solution. The reaction was allowed to stir for 30 minutes. At this time, the reaction was concentrated in vacuo, and then Et_2O was added to the mixture, followed by concentrating the mixture in vacuo (repeat 3x total) to provide a light-yellow solid. Anal. Calcd for $C_{28}H_{43}N_3O$·2.00 tartaric acid·1.50 H_2O: C, 56.53; H, 7.64; N, 5.49; found: C, 56.91; H, 7.83; N, 5.12.

cis-(R)-1-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)pyrrolidin-3-ol (**29**). See General Procedure A. Conditions: aldehyde **7** (97.0 mg, 0.275 mmol, 1.00 equiv.), pyrrolidin-3-ol (47.9 mg, 0.550 mmol, 2.00 equiv), glacial AcOH (36.2 μL, 0.633 mmol, 2.30 equiv), STAB (134 mg, 0.633 mmol, 2.30 equiv), DCE (2.75 mL, 0.10M). The crude product was purified via flash chromatography using 5:95:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide indole **29** as a light-yellow foam (112 mg, 97% yield). R_f = 0.20 (5:95:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 6.0 Hz, 1H), 7.36 (d, *J* = 6.0 Hz, 1H), 7.21 (m, 2H), 7.11 (t, *J* = 5.1 Hz, 1H), 4.32 (m, 1H), 4.18 (m, 1H), 3.86 (s, 2H), 3.18 (d, *J* = 9.0 Hz, 2H), 2.93 (m, 1H), 2.74 (m, 1H), 2.60 (m, 1H), 2.43-1.98 (m, 10H), 1.78-1.52 (m, 8H), 1.57 (m, 2H), 1.15 (m, 1H), 0.90 (d, *J* = 5.1 Hz, 6H); MS(ESI) *m/z*: 424.6 [M+H]+. The bis tartrate salt of amine **29** was formed by dissolving the amine in MeOH (0.10M) at room temperature. To the reaction was added L-(+)-tartaric acid (2.50 equiv) in a MeOH solution. The reaction was allowed to stir for 30 minutes. At this time, the reaction was concentrated in vacuo, and then Et_2O was added to the mixture, followed by concentrating the mixture in vacuo (repeat 3x total) to provide a tan solid. Anal. Calcd for $C_{27}H_{41}N_3O$·2.50 tartaric acid·2.00 H_2O: C, 53.23; H, 7.24; N, 5.03; found: C, 53.60; H, 6.96; N, 4.95.

cis-1-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)azetidin-3-ol (**30**). See General Procedure A. Conditions: aldehyde **7** (40 mg, 0.113 mmol, 1.00 equiv.), azetidin-3-ol·HCl (24.8 mg, 0.226 mmol, 2.00 equiv), *i*Pr_2NEt (45.3 μL, 0.260 mmol, 2.30 equiv), glacial AcOH (14.9 μL, 0.260 mmol, 2.30 equiv), STAB (55.1 mg, 0.260 mmol, 2.30 equiv), DCE (1.10 mL, 0.10M). The crude product was purified via flash chromatography using 5:95:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide indole **30** as a light-golden oil (37.4 mg, 81%

yield). R_f = 0.15 (5:95:1 MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 5.7 Hz, 1H), 7.58 (br, 1H), 7.36 (d, *J* = 6.3 Hz, 1H), 7.21 (m, 2H), 7.12 (t, *J* = 5.4 Hz, 1H), 4.44 (p, *J* = 4.2 Hz, 1H), 4.19 (m, 1H), 3.84 (s, 2H), 3.69 (m, 2H), 3.18 (d, *J* = 9.0 Hz, 2H), 3.07 (m, 2H), 2.36-2.22 (m, 3H), 2.10-1.88 (m, 4H), 1.78-1.46 (m, 7H), 1.40 (m, 2H), 1.14 (m, 1H), 0.90 (d, *J* = 5.1 Hz, 6H); MS(ESI) *m/z*: 410.6 [M+H]+. The bis tartrate salt of amine **30** was formed by dissolving the amine in MeOH:CH_2Cl_2 (3:2, 0.10M) at room temperature. To the reaction was added L-(+)-tartaric acid (2.00 equiv) in a MeOH solution. The reaction was allowed to stir for 30 minutes. At this time, the reaction was concentrated in vacuo, and then Et_2O was added to the mixture, followed by concentrating the mixture in vacuo (repeat 3x total) to provide an off-white solid. Anal. Calcd for $C_{26}H_{39}N_3O$·2.00 tartaric acid·2.00 H_2O: C, 54.75; H, 7.43; N, 5.63; found: C, 54.92; H, 7.27; N, 5.35.

cis-2-(((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)amino)ethan-1-ol (**31**). See General Procedure A. Conditions: aldehyde **7** (100 mg, 0.284 mmol, 1.00 equiv), ethanolamine (17.0 μL, 0.284 mmol, 1.00 equiv), Note: glacial AcOH not used, STAB (180 mg, 0.851 mmol, 3.00 equiv), DCE (6.00 mL, 0.050M). The crude product was purified via flash chromatography using 8:92:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide indole **31** as a liquid (25 mg, 22% yield). R_f = 0.30 (10:90 MeOH:CH_2Cl_2, UV, I_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, *J* = 5.7 Hz, 1H), 7.36 (d, *J* = 6.3 Hz, 1H), 7.18 (t, *J* = 5.1 Hz, 2H), 7.06 (d, *J* = 5.7 Hz, 1H), 4.21 (m, 1H), 3.66 (br s, 1H), 3.22 (s, 1H), 2.79 (s, 1H), 2.32 (br s, 3H), 2.10 (s, 4H), 1.72-1.61 (m, 7H), 1.43-1.32 (m, 2H), 1.26 (s, 2H), 1.15 (s, 1H), 0.89 (d, *J* = 4.8 Hz, 6H); MS(ESI) *m/z*: 398.24 [M+H]+. HRMS Calcd for $C_{25}H_{39}N_3O$ (M+H)+ 398.3093, found 398.3173.

cis-2-amino-5-guanidino-N-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)pentanamide (**33**). Z-Arg-OH (0.39 g, 0.68 mmol, 1.20 equiv), HOBT (0.099 g, 0.65 mmol, 1.15 equiv), and triethylamine (0.110 mL, 0.79 mmol, 1.40 equiv), were added to 10 mL of CH_2Cl_2 (0.057M) at room temperature and stirred until the entire solid dissolved. The reaction was cooled in an icebath at 5˚C and EDCI (0.13 g, 0.68 mmol, 1.20 equiv) was added and stirred at 5˚C for 2 hours. At this stage, a solution of amine **9** (0.20 g, 0.57 mmol, 1.00 equiv) in 5 mL of CH_2Cl_2 was added to the reaction, and then the reaction was allowed to warm to room temperature and was monitored by TLC (10:90:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.)). After 2 hours, the reaction was complete by TLC. The reaction was quenched with saturated $NaHCO_3$ (aq.), stirred 5 minutes, and the layers were then separated. The aqueous layer was extracted 2x with CH_2Cl_2, the organic layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a crude residue that was purified via flash chromatography using 3:96:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide Cbz intermediate **32** as a white solid (0.35 g, 67% yield). R_f = 0.50 (10:90:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2).

To a solution of Cbz intermediate **32** (150 mg, 0.160 mmol, 1.00 equiv) in THF (1.60 mL, 0.10M), was added 10% Pd/C (15.0 mg, 10 wt%), and then the reaction was fitted with a 3-way adapter containing a H_2 (g) balloon. The reaction atmosphere was purged, and then backfilled with H_2 (g). This was repeated 3x total. The reaction was stirred at room temperature and monitored by TLC. After 3 hours, TLC showed full consumption of the starting material. The reaction was poured over a pad of Celite, filtered, and the Celite was washed thoroughly with THF. The resulting filtrate was concentrated in vacuo, and the residue was purified by trituration with CH_2Cl_2 and Et_2O to provide amide **33** as a white solid (73 mg, 87% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.32 (s, 2H), 7.72 (br s, 1H), 7.50 (d, *J* = 6.0 Hz, 1H), 7.44 (d, *J*

= 6.3 Hz, 1H), 7.41 (s, 1H), 7.08 (t, *J* = 6.0 Hz, 1H), 6.95 (t, *J* = 5.7 Hz, 1H), 4.39 (dd, *J* = 10.8, 3.6 Hz, 1H), 4.31-4.24 (m, 2H), 3.83 (br s, 1H), 3.07 (br s, 3H), 2.90 (m, 1H), 2.66 (s, 2H), 2.28 (d, *J* = 13.5, 2H), 2.16 (t, *J* = 8.4 Hz, 3H), 1.91-1.85 (m, 4H), 1.70 (br s, 2H), 1.54 (br s, 4H), 1.41-1.35 (m, 4H), 1.12 (m, 1H), 0.85 (d, *J* = 4.8 Hz, 6H); MS(ESI) *m/z*: 510.36 [M+H]+. Anal. Calcd for $C_{29}H_{47}N_7O \cdot 2.00\ CH_2Cl_2$: C, 54.79; H, 7.56; N, 14.43; found: C, 54.89; H, 7.31; N, 14.46.

cis-6-amino-N-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)hexanamide (**35**). 6-(((benzyloxy)carbonyl)amino)hexanoic acid (0.18 g, 0.68 mmol, 1.20 equiv), HOBT (0.099 g, 0.65 mmol, 1.15 equiv), and triethylamine (0.110 mL, 0.79 mmol, 1.40 equiv) were added to 10 mL of CH_2Cl_2 (0.057 M) at room temperature and stirred until the entire solid dissolved. The reaction was then cooled in an icebath at 5˚C and EDCI (0.13 g, 0.68 mmol, 1.20 equiv) was added and stirred at 5˚C for 2 h. At this stage, amine **9** (0.20 g, 0.57 mmol, 1.00 equiv) was dissolved in 5 mL of CH_2Cl_2, and added to the reaction at 5˚C. The reaction was stirred at 5˚C for 1 hour, and then it was allowed to warm to room temperature and stir for an additional 12 hours. The reaction was quenched with saturated $NaHCO_3$ (aq.), stirred 5 minutes, and the layers were then separated. The aqueous layer was extracted 2x with CH_2Cl_2, the organic layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a crude residue that was purified via flash chromatography using 3:96:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide carbamate intermediate **34** as an oil (0.30 g, 88 % yield). R_f = 0.50 (10:90:3 drops MeOH:CH_2Cl_2:NH_4OH (aq.), UV, I_2).

To a solution of Cbz intermediate **34** (140 mg, 0.23 mmol, 1.00 equiv) in 2.30 mL of THF (0.10M), was added 10% Pd/C (14.0 mg, 10 wt%), and then the reaction was fitted with a 3-way adapter containing a H_2 (g) balloon. The reaction atmosphere was purged, and then

backfilled with H_2 (g). This was repeated 3x total. The reaction was stirred at room temperature and monitored by TLC. After 3 hours, TLC showed full consumption of the starting material. The reaction was poured over a pad of Celite, filtered, and the Celite was washed thoroughly with THF. The resulting filtrate was concentrated in vacuo, and the residue was purified by trituration with CH_2Cl_2 and Et_2O to provide amide **35** as a white solid (42 mg, 39% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (d, *J* = 7.5 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 5.1 Hz, 2H), 7.10 (t, *J* = 7.5 Hz, 1H), 5.93 (s, 1H), 4.59 (d, *J* = 5.1 Hz, 2H), 4.21 (m, 1H), 3.57 (t, *J* = 5.4 Hz, 4H), 3.22 (d, *J* = 11.4 Hz, 2H), 2.56-2.50 (m, 3H), 2.43-2.32 (m, 2H), 2.17 (t, *J* = 7.5 Hz, 2H), 2.12 (s, 2H), 1.78-1.51 (m, 11H), 1.45-1.31 (m, 4H), 1.29-1.16 (m, 1H), 0.90 (d, *J* = 6.6 Hz, 6H); MS(ESI) *m/z*: 467.30 [M+H]+. The bis HCl salt of amine **35** was formed by dissolving the amine in CH_2Cl_2 (0.10M) at room temperature, followed by the addition of HCl (2.0M in Et_2O, 2.20 equiv). The reaction was allowed to stir for 10 minutes. At this time, the reaction was concentrated in vacuo, Et_2O was added to the mixture, followed by concentrating the mixture in vacuo (repeat 3x total) to provide the HCl salt of amine **35**. Anal. Calcd for $C_{29}H_{46}N_4O$·2.00 HCl·2.00 CH_2Cl_2·2.00 Et_2O·1.00 H_2O: C, 53.49; H, 8.52; N 6.40; found: C, 53.73; H, 8.39; N, 6.20.

cis-2-(ethylamino)-N-((1-(1-(-4-isopropylcyclohexyl)piperidin-4-yl)-1H-indol-3-yl)methyl)acetamide (**37**). Z-glycine-OH (0.20 g, 0.67 mmol, 1.20 equiv), HOBT (0.099 g, 0.65 mmol, 1.15 equiv), and triethylamine (0.110 mL, 0.79 mmol, 1.40 equiv) were added to 10 mL CH_2Cl_2 (0.057 M) and stirred until the entire solid dissolved. The reaction was then cooled in an icebath at 5˚C and EDCI (0.13 g, 0.68 mmol, 1.20 equiv) was added and stirred at 5˚C for 2 h. At this stage, amine **9** (0.20 g, 0.57 mmol, 1.00 equiv) was dissolved in 5 mL of CH_2Cl_2, and added to the reaction at 5˚C. The reaction was allowed to warm to room temperature

and stir for an additional 2 hours. The reaction was quenched with saturated $NaHCO_3$ (aq.), stirred 5 minutes, and the layers were then separated. The aqueous layer was extracted 2x with CH_2Cl_2, the organic layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to provide a crude residue that was purified via flash chromatography using 1:98:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide Cbz intermediate **36** (0.21 g, 68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, *J* = 5.7 Hz, 1H), 7.36 (d, *J* = 6.3 Hz, 1H), 7.32 (s, 5H), 7.24 (m, 2H), 7.11 (t, *J* = 5.7 Hz, 1H), 6.11 (s, 1H), 5.45 (s, 1H), 5.08 (s, 2H), 4.19 (m, 1H), 3.85 (d, *J* = 4.2 Hz, 2H), 3.22 (d, *J* = 8.7 Hz, 2H), 2.44 (s, 1H), 2.31 (d, *J* = 7.8 Hz, 2H), 2.09 (t, *J* = 7.8 Hz, 4H), 1.73 (br s, 2H), 1.65 (m, 3H), 1.43-1.38 (m, 2H), 1.17 (m, 1H), 0.89 (d, *J* = 4.8 Hz, 6H); MS(ESI) *m/z*: 545.28 [M+H]+.

To a solution of Cbz intermediate **36** (0.15 g, 0.308 mmol, 1.00 equiv) in 3.1 mL of absolute ethanol (0.10M), was added 10% Pd/C (15.0 mg, 10 wt%), and then the reaction was fitted with a 3-way adapter containing a H_2 (g) balloon. The reaction atmosphere was purged, and then backfilled with H_2 (g). This was repeated 3x total. The reaction was stirred at room temperature and monitored by TLC. After 4 hours, TLC showed full consumption of the starting material. The reaction was poured over a pad of Celite, filtered, and the Celite was washed thoroughly with ethanol. The resulting filtrate was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel, using 5:95:1 MeOH:CH_2Cl_2:NH_4OH (aq.) to provide ethylated amine **37** as an oil (55.2 mg, 41% yield). R_f = 0.50 (10:90 MeOH: CH_2Cl_2:NH_4OH (aq.), UV, I_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (t, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 4.8 Hz, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 5.31 (s, 1H), 4.60 (d, *J* = 5.4 Hz, 2H), 4.32 (br s, 1H), 3.38 (m, 4H), 2.71-2.44 (m, 9H), 2.11 (d, *J* = 11.7 Hz, 2H), 1.88-1.64 (m, 5H), 1.40 (dd, *J* = 10.2, 13.2 Hz, 2H), 1.28-1.20 (m, 1H), 1.07 (t, *J* = 6.9 Hz, 3H), 0.92 (d, *J* =

6.3 Hz, 6H); MS(ESI) *m/z*: 439.28 [M+H]+. Anal. Calcd for $C_{27}H_{42}N_4O \cdot 0.50\ H_2O \cdot 0.50\ CH_2Cl_2$: C, 67.39; H, 9.05; N, 11.43; found: C, 67.02; H, 9.01; N, 11.69.

In vitro pharmacological Characterization

Cells. Human NOP, mu, delta, and kappa opioid receptors were individually expressed in Chinese hamster ovary cells stably transfected with the human receptor cDNA, as we have described previously.[24, 32] Kappa-CN cells were used for KOP radioligand binding assays, while Kappa-FLG19 cells were used in KOP [^{35}S]GTPγS functional assays. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum, in the presence of 0.4 mg/ml G418 and 0.1% penicillin/streptomycin, in 100-mm plastic culture dishes.

Membrane preparation. The cell lines are grown to full confluency, then harvested for membrane preparation. The membranes are prepared in 50 mM Tris buffer (pH 7.4). Cells are scraped and centrifuged at 500 × g for 12 mins. The cell pellet is homogenized in 50 mM Tris with a Fisher Scientific PowerGen 125 rotor-stator type homogenizer, centrifuged at 20,000 × g for 25 mins, washed and recentrifuged once more and aliquoted at a concentration of 3 mg/mL protein per vial and stored in a -80˚C freezer till further use.

Receptor Binding. The assay is performed in a 96–well polystyrene plate using triplicates of six concentrations of each test compound and tritiated ligands [^{3}H]DAMGO (48 Ci/mmol, K_d 1.6 nM for MOP), [^{3}H]DPDPE (46 Ci/mmol, K_d 1.9 nM for DOP), [^{3}H]U69593 (41.7 Ci/mmol, K_d 2.1 nM for KOP), or [^{3}H]N/OFQ (130 Ci/mmol, K_d 0.2 nM for NOP). Nonspecific binding was determined by using 1.0 μM of the unlabeled nociceptin for NOP, 10 μM unlabeled DAMGO for MOP, 10 μM unlabeled DPDPE for DOP, and 10 μM unlabeled U69,593 for KOP. Assays were initiated by addition of membrane homogenates and samples were incubated for 60 min at 25˚C in a total volume of 1.0 mL. In NOP receptor experiments, 1 mg/mL BSA is added

to the assay buffer. The amount of protein in the binding assay was 15 μg. The incubation was terminated by rapid filtration through 0.5% PEI-soaked glassfiber filter mats (GF/C Filtermat A, Perkin-Elmer) on a Tomtec Mach III cell harvester and washed 5 times with 0.5 mL of ice-cold 50 nM Tris-HCl, pH 7.4 buffer. The filters were dried overnight and soaked with scintillation cocktail before counting on a Wallac Beta plate 1205 liquid scintillation counter. Radioactivity was determined as counts per minutes (cpm). IC_{50} values were determined by using at least six concentrations of each compound, and calculated using GraphPad Prism 6. (ISI, San Diego, CA). K_i values were determined by the method of Cheng and Prusoff. Each compound data is from at least three independent experiments conducted in triplicate. Statistical analysis was conducted using GraphPad Prism.

[^{35}S]GTPγS Functional assay. The efficacy of the compounds were determined by the ability to stimulate [^{35}S]GTPγS binding to cell membranes, and compared to standard agonists. The functional assay was conducted in Buffer A, containing 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10 mM magnesium chloride (MgCl2) 100 mM sodium chloride (NaCl) at pH 7.4. Membrane prepared as described above was incubated with [^{35}S]GTPγS (150,000 cpm/well), guanosine diphosphate (GDP) (10 mM), and the test compound, in a total volume of 1 ml for 60 minutes at 25˚C. Samples were filtered over Filtermat A and counted as described for the binding assays. A dose response curve with a prototypical full agonist at the respective receptor was conducted in each experiment to identify full and partial agonist compounds. Typically, the standard full agonists (N/OFQ for NOP, DAMGO for MOP, U69593 for KOP, and DPDPE DOP) showed at least 2-fold to 5-fold stimulation over basal. The EC_{50} values are calculated using GraphPad Prism 6 (ISI, San Diego,

CA) The % stimulation by the test compound is calculated as the percentage of the standard full agonist stimulation taken as 100% (Table 1).

Caco-2 intestinal permeability assay and the MDR-MDCK cell permeability assay

The data shown in Table 2 are from experiments performed by a commercial service and were determined by the standard Caco-2 two-way permeability analysis with monitoring of test compound by HPLC/MS/MS. Concentration of test compound on both apical side (A → B) and basolateral side (B → A) were determined as the apparent permeability $P_{app}(10^{-6}$ cm/s). The MDR-MDCK cell permeability also measured using a two-way permeability measurement, with monitoring of test compound by HPLC/MS/MS.

Human and rat/mouse liver microsomal stability assays

These assays were performed by a commercial service using a standard protocol. Briefly, 1 μM test compounds in 100 mM potassium phosphate, pH 7.4, 5 mM magnesium chloride were incubated at 37˚C in a shaking water bath after initiation of reaction by the addition of 1 mM NADPH. Aliquots taken at time points 30, 45, and 60 min. The reactions were terminated by addition of an equal volume of acetonitrile containing 0.1% formic acid, and samples processed and analysed by LC-MS to monitor loss of parent compound. Percent of remaining parent compound is calculated relative to t = 0 min, and assays were conducted in duplicate. The validation control (testosterone for human and rodent) was assayed by LC-MS/MS using electrospray ionization. The peak area response ratio to internal standard propranolol at each time point was compared to the response ratio at time 0 to determine the percent remaining. Graph Pad (San Diego, CA) was used to calculate the half-life, and data were fit to a single-phase exponential decay equation.

In vivo pharmacokinetics and brain penetration of selected compounds in rats

Male Sprague-Dawley rats (250–300 g) were administered a single dose of the test compound in the formulated vehicle via subcutaneous (s.c) injection. Dosing volume and dose were 2 mL/kg and 3 mg/kg, respectively for all compounds tested. Compounds were formulated as a 1.5 mg/ml solution in 5% dimethylacetamide / 95% of 30% SBECD (betacyclodextrin sulfobutyl ether). The following sampling times were used (n = 3/time point): 0.5, 1, 2, 4, 8 h after dosing. Rats were lightly anesthetized with CO_2, blood was collected via cardiac puncture, and brains were removed from the cranium. Blood samples were collected into vials containing K2-EDTA, and vials were capped and temporarily stored on ice. Brain samples were temporarily put on dry ice. Plasma was separated from blood by centrifugation and frozen at -80°C until bioanalysis. Brains were rinsed with ice-cold phosphate buffered saline, blotted dry, weighed, and stored at -80°C until bioanalysis.

Bioanalytical method. Plasma samples were thawed on ice, and a 50-µl aliquot was transferred to a 1-ml plastic tube. 5 µl MeOH was added to the samples. 200 µl of 50 ng/ml internal standard (terfenadine) in MeOH / acetonitrile (1:1, v/v) was added to the plasma, vortexed, and centrifuged (15 mins at 4000 rpm). The resultant supernatants were diluted 10x with MeOH/water (1:1, v/v, containing 0.1% formic acid) for injection and analyzed. Brain samples were thawed on ice, placed into plastic tubes, and 4 volumes of purified water per gram of brain was added (1:4, w/v). Brains were homogenized using a mechanical high speed tissue homogenizer; 50 µl of brain homogenate was transferred to a 1-ml plastic tube. 5 µl MeOH was added to the samples. 200 µl of 50 ng/ml internal standard (terfenadine) in MeOH / acetonitrile (1:1, v/v) was added, vortexed, and centrifuged (15 mins at 4000 rpm). The resultant

supernatants were diluted 10x with MeOH/water (1:1, v/v, containing 0.1% formic acid) for injection and analyzed.

Processed plasma and brain samples were analyzed by liquid chromatography-tandem mass spectrometry (LC/MS/MS) on an AB Sciex API 4000 (Applied Biosystems, CA). The LC separation used a binary gradient and C18 reverse phase column [Kinetex C18 100A, 30 × 3 mm). The column was maintained at room temperature. Mobile phase A consisted of 0.05% formic acid in 5 mM aqueous NH_4OAc and mobile phase B consisted of 0.1% formic acid in acetonitrile. The flow rate was held constant at 0.7 ml/min. Detection was using electrospray ionization operating in positive ion mode.

Pharmacokinetic data analysis. The noncompartmental plasma and brain pharmacokinetics were determined using the program WinNonlin version 4.0.1 (Pharsight, Mountain View, CA). The area under the curve plasma concentration-time profile was established by linear trapezoidal rule. The terminal phase half-life was calculated using at least three data points in the terminal phase excluding the Cmax.

In vivo efficacy in the 6-OH hemilesioned rat model of Parkinson's disease

Animal Subjects

Experiments were performed in accordance with the ARRIVE guidelines. Experimenters were blinded to treatments. Male Sprague-Dawley rats (150 g, 6 week old; Envigo, S. Pietro al Natisone, Italy) were housed in a standard facility with free access to food (4RF21 standard diet; Mucedola, Settimo Milanese, Milan, Italy) and water, and kept under regular lighting conditions (12 hr dark/light cycle). Animals were housed in groups of 5 for a 55x33x20 cm polycarbonate cage (Tecniplast, Buguggiate, Varese, Italy) with a Scobis Uno bedding (Mucedola, Settimo Milanese, Milan, Italy) and environmental enrichments. The experimental protocols were

approved by the Italian Ministry of Health (license n. 170/2013-B). Adequate measures were taken to minimize animal pain and discomfort. At the end of the experiments, rats were sacrificed with an overdose of isoflurane.

Unilateral 6-OHDA lesion

The unilaterally 6-OHDA lesioned rat, the most popular and best validated model of PD,[33, 34] was used to assess the ability of NOP partial agonists to improve parkinsonian-like motor deficits. Unilateral lesion of dopaminergic neurons was induced under isoflurane anaesthesia as previously described.[10, 16] Eight micrograms of 6-OHDA freebase (dissolved in 0.9% saline solution containing 0.02% ascorbic acid) were stereotaxically injected in the medial forebrain bundle according to following coordinates from bregma: antero-posterior= -4.4 mm, medio-lateral= -1.2, dorso-ventral=-7.8 mm below dura.[35] Animals were pre-treated with antibiotics (Synulox™, 50 µL/kg, i.p.). The wound was sutured and infiltrated with 2% lidocaine solution (Esteve™). In order to select rats that were successfully hemilesioned, two weeks after 6-OHDA injection, rats were injected with a test dose of D-amphetamine (5 mg/kg, i.p., dissolved in saline), and those performing >7 turns per minute in the direction ipsilateral to the lesion were enrolled in the study. In fact, this behaviour has been associated with >95% loss of striatal dopaminergic terminals[16] and extracellular dopaminergic levels.[36]

Behavioral Tests

Bar Test. The bar test,[10] also known as catalepsy test,[37] measures the ability of the animal to react to an externally imposed position. The right and left forepaws were alternatively placed on three blocks of increasing heights (3, 6, 9 cm). The immobility time (in sec) of each forepaw on the blocks was recorded (the cut-off for each step was set at 20 sec).

Drag Test. The drag test, modification of the "wheelbarrow test",[38] measures the ability of the animal to balance its body posture using the forelimbs, in response to backward dragging. Each rat was gently lifted from the abdomen leaving the forepaws on the table and dragged backwards at a constant speed of 20 cm/sec for a fixed distance of 1 m. Two different observers counted the number of touches made by each forepaw.

Rotarod Test. This test measures the ability of the animal to run on a rotating cylinder and provides different information on a variety of motor parameters such as coordination, balance, muscle tone, gait and motivation to run.[39] The fixed-speed rotarod test was employed using a previously validated protocol.[10, 31] Animals were tested starting from 5 rpm, speed was stepwise increased by 5 rpm every 180 sec, and total time spent on the rod was calculated.

Experimental protocols and design

Motor behavior in 6-OHDA hemilesioned, L-DOPA naïve rats

Hemilesioned rats which met the selection criteria after amphetamine testing after 2 weeks were used in the behavioral tests. Ten days later, these rats were subjected to the bar, drag and rotarod tests repeated in a fixed sequence as training. When motor performance was reproducible (usually after 7-10 days), rats were randomized and treated with the respective doses of the test partial agonist, L-DOPA or vehicle. Rats were tested 3-4 times, and a 3-day washout was allowed between treatments. In the bar and drag test, motor activity was assessed 20 and 90 min after compound administration both at the contralateral and ipsilateral paw, and expressed as absolute values for the bar and drag tests, and as percentage of the control session for the rotarod test. Since no motor changes were observed at the ipsilateral paw after compound administration, data for the ipsilateral paw not shown.

Statistical analysis

Statistical analysis was performed using PRISM 6.0 (GraphPad Software Inc., San Diego, CA) with two-way ANOVA followed by the Bonferroni test. Rotarod performance was expressed as % of control time on rod and analyzed with two-way ANOVA followed by Bonferroni test. Statistical significance was set at $p<0.05$.

ASSOCIATED CONTENT

Supporting Information

SMILES data (Molecular formula strings) for all final compounds along with their in vitro pharmacological data are provided.

AUTHOR INFORMATION

Corresponding Author

Dr. Nurulain T. Zaveri, Tel: 650-254-0786. Email: nurulain@astraeatherapeutics.com

Author Contributions

U.G.K. and M.E.M. contributed equally to this work.

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ABBREVIATIONS USED

DOP, delta opioid receptor; GPCR, G-protein coupled receptor; KOP, kappa opioid receptor; MOP, mu opioid receptor; NOP, N/OFQ opioid peptide receptor; N/OFQ, nociceptin/orphaninFQ; L-DOPA, levodopa/l-dihydroxyphenylalanine; LID, levodopa-induced dyskinesia.

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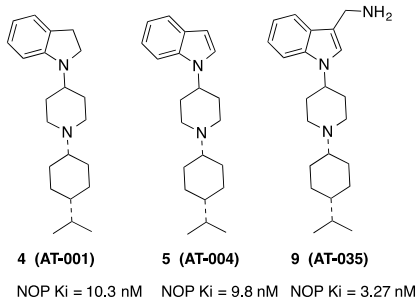
TOC GRAPHIC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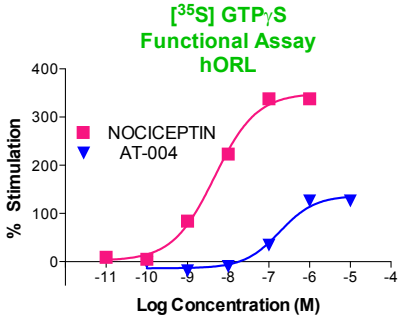
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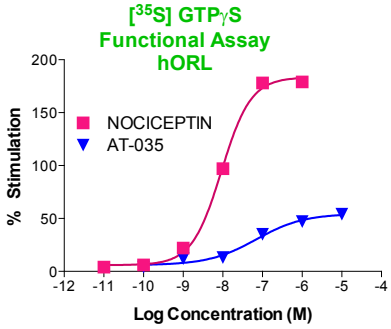
Log Concentration (M)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% Stimulation
Log Concentration (M)