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In Vivo Characterization of a Dual Adenosine A_{2A}/A_1 Receptor Antagonist in Animal Models of Parkinson's Disease

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The in vivo characterization of a dual adenosine A_{2A}/A_1 receptor antagonist in several animal models of Parkinson's disease is described. Discovery and scale-up syntheses of compound **1** are described in detail, highlighting optimization steps that increased the overall yield of **1** from 10.0% to 30.5%. Compound **1** is a potent A_{2A}/A_1 receptor antagonist in vitro (A_{2A} $K_i = 4.1$ nM; A_1 $K_i = 17.0$ nM) that has excellent activity, after oral administration, across a number of animal models of Parkinson's disease including mouse and rat models of haloperidol-induced catalepsy, mouse model of reserpine-induced akinesia, rat 6-hydroxydopamine (6-OHDA) lesion model of drug-induced rotation, and MPTP-treated non-human primate model.

Introduction

Parkinson's disease (PD[a]) is a chronic, progressive neurological disease that affects ~1% of the population over the age of 65.[1] It is characterized by progressive impairment in motor function that is often accompanied by disturbances in mood and cognitive function. Most of the motor impairments of PD are caused by a gradual loss of dopamine (DA) producing neurons in the ventral midbrain and concomitant loss of DA input to forebrain (striatal) motor structures.[2,3] The loss of DA input to the neostriatum leads to dysregulation of striatal function and the classic motor symptoms of PD, such as resting tremor, muscular rigidity, and bradykinesia.

Most of the treatments aim to restore dopamine signaling and thereby reduce the severity of the motor symptoms. Dopamine replacement therapy using L-DOPA, the precursor to dopamine, remains the gold-standard treatment for PD. Other approaches include inhibition of DA turnover using monoamine oxidase type B (MAO-B) inhibitors,[4] catechol *O*-methyltransferase (COMT) inhibitors,[5] and inhibition of dopamine reuptake[6] or direct agonists[7] of postsynaptic dopamine receptors. Although the dopamine targeted therapies work well to address the PD related motor disturbances, they all produce undesirable side effects (dyskinesia, hallucinations, on−off effects) that become more severe and problematic with continued treatment. Also, the aforementioned therapies typically show reduced efficacy as motor functions deteriorate and the disease progresses. Moreover, these treatments do not alter disease progression and do not address the mood, postural instability, or cognitive disturbances that frequently accompany PD.

As a result of the limitations of dopamine replacement agents, drug companies have sought non-dopamine based treatment for PD. One non-dopaminergic approach that has received considerable attention is modulation of adenosine receptors.[8] Adenosine is a neuromodulator that coordinates responses to dopamine and other neurotransmitters in areas of the brain that are responsible for motor function, learning and memory.[9] Adenosine comprises four distinct receptor subtypes designated A_1, A_{2A}, A_{2B}, and A_3 belonging to the G-protein-coupled receptor superfamily.[10] Adenosine A_1 and A_3 receptors are coupled to inhibitory G proteins, while A_{2A} and A_{2B} receptors are coupled to stimulatory G proteins. On the basis of receptor autoradiography in rodents, the greatest densities of A_{2A} receptors are found in the striatum[11] which closely matches the distribution in humans based on PET imaging.[12]

As described above, loss of dopamine input into the neostriatum is a hallmark of PD and underlies many of the cardinal motor symptoms of this disorder. In the striatum adenosine A_{2A} receptors colocalize and physically associate with dopamine D_2 receptors.[2,3] A_{2A} and D_2 receptors have opposing effects on adenylate cyclase and cAMP production in cells such that activation of A_{2A} receptors inhibits dopamine D_2 receptor signaling. Conversely, A_{2A} receptor antagonists enhance D_2 dependent signaling as shown by induction of immediate early gene c-fos expression in the striatopallidal pathway[13] and facilitate other D_2 mediated responses. Of importance to PD, pharmacological blockade of A_{2A} receptors has shown dramatic beneficial effects in preclinical animal models of PD, showing potentiation of dopamine-mediated responses in dopamine depleted (6-hydroxydopamine treated) animals and dramatic relief of parkinsonian symptoms in MPTP-treated nonhuman primates.[14] A_{2A} antagonists facilitate dopamine receptor signaling and thereby normalize motor

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[a] Abbreviations: 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; DA, dopamine; L-DOPA, levodopamine, MAO-B, monoamine oxidase type B; COMT, catchol *O*-methyltransferase; cAMP, cyclic adenosine monophosphate; PK, pharmacokinetics.

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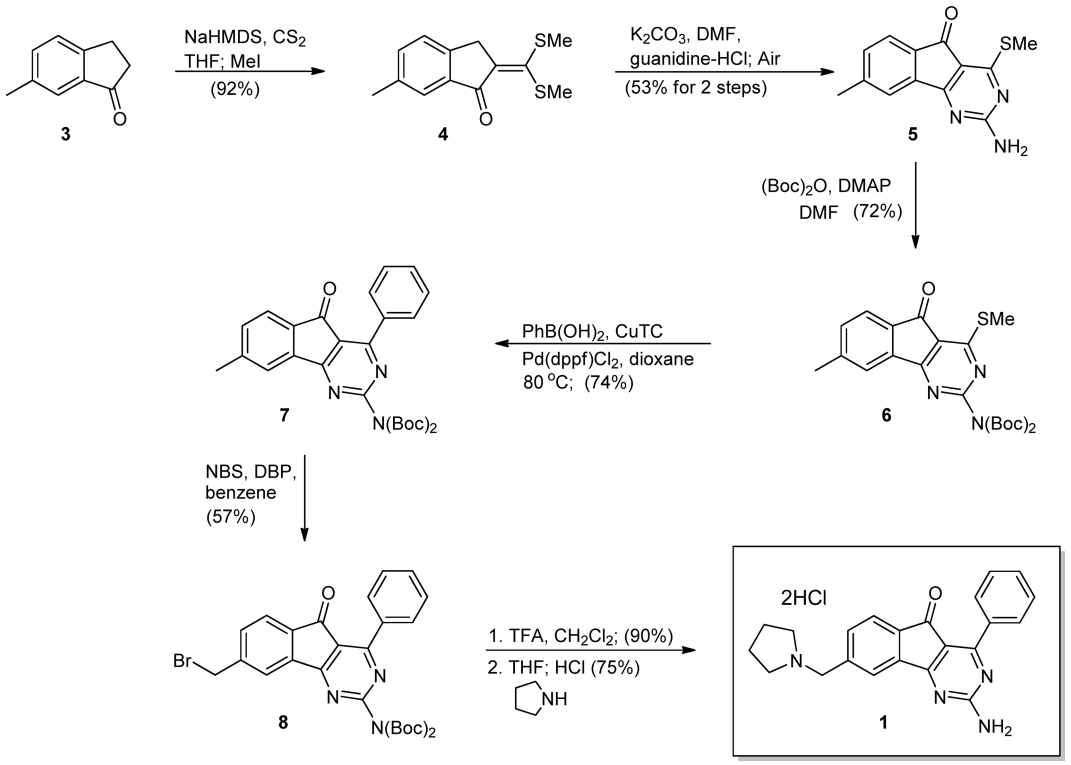
Scheme 1. Discovery Synthesis of **1**



function in animal models of dopamine dysregulation. As a result of these findings, the adenosine A_{2A} receptor has become a sought after target for treating PD. Blockade of A_{2A} signaling by selective A_{2A} receptor antagonists (e.g., **2** (KW-6002),[15] istradefylline) was shown to be beneficial for not only enhancing the therapeutic effects of L-DOPA but also reducing dyskinesia from long-term L-DOPA treatment.[16]

The adenosine A_1 receptor is also expressed in the striatum. On the basis of anatomical and in vivo microdialysis studies, A_1 receptors appear to be localized presynaptically of dopamine axon terminals where they inhibit dopamine release.[17] A_1 receptor antagonists facilitate dopamine release in the striatum and, like A_{2A} receptors, potentiate dopamine mediated responses. Antagonism of both the A_{2A} and A_1 would be synergistic; inhibition of the A_1 receptor will facilitate dopamine release, while inhibition of the A_{2A} receptor will enhance postsynaptic responses to dopamine. Interestingly, the A_1 receptor is also concentrated in neocortical and limbic system structures that are important for cognitive function. Pharmacological inhibition of A_1 receptors enhances neurotransmitter release in the hippocampus[18] and enhances performance in animal models of learning and memory.[19]

The major unmet medical needs of PD are improved symptomatic treatment without inducing adverse effects (primarily dyskinesia) associated with long-term L-DOPA or dopamine agonist therapy; opportunity to slow disease progression by protecting midbrain dopamine and other neurons from degeneration; treatment of disease comorbidities, including cognitive dysfunction, anxiety, and depression. On the basis of published preclinical and human clinical data, a dual A_{2A}/A_1 receptor antagonist may address many of these unmet needs.

Results and Discussion

We recently published a series of arylindenopyrimidines as dual A_{2A}/A_1 receptor antagonists that reverse haloperidol induced catalepsy in mice when dosed orally.[20] The compounds typically showed very potent in vitro activity for both A_{2A} and A_1 and had A_1/A_{2A} ratios ranging from ~1 to 10. Compound **1** was identified as an ideal dual A_{2A}/A_1 receptor antagonist having excellent functional in vitro (A_{2A} K_i = 4.1 nM; A_1 K_i = 17.0 nM) and in vivo activity and showed excellent beneficial effects in several preclinical animal models of PD across a number of different species. We report herein the in vivo characterization of **1** in animal models of PD including mouse[21] and rat[22] models of neuroleptic-induced catalepsy, mouse model of reserpine-induced akinesia,[23] rat 6-hydroxydopamine (6-OHDA) lesion model of drug-induced rotation,[24] and MPTP-treated non-human primate model.[25]

The discovery synthesis of compound **1** started with the commercially available 6-methylindanone **3** which was deprotonated with NaHMDS and reacted with CS_2 followed by MeI to afford the dithioketenacetal **4** (Scheme 1).[26] Compound **4** reacts with guanidine to form an intermediate aminopyrimidine, not shown or isolated, that is then oxidized to the corresponding ketone by passing air through the solution to give **5**.[27] The aminopyrimidine was protected using excess $(Boc)_2O$ and 4-dimethylaminopyridine (DMAP) to give **6**. Efforts to mono-Boc compound **5** were unsuccessful as the di-Boc compound was forming while starting material was present. Also, the reaction was very sluggish without the addition of DMAP. The phenyl substituent was installed via a modified Suzuki reaction under Liebeskind-type conditions using the methylthioether as the coupling partner to afford **7**.[28] The Suzuki reaction proceeded smoothly with the di-Boc protected aminopyrimidine but could not be accomplished on the unprotected aminopyrimidine **5** under the same conditions. Compound **7** underwent benzyl bromination to afford the corresponding bromide **8**. Removal of the Boc groups with TFA followed by alkylation with pyrrolidine gave the desired

Scheme 2. Optimized Large Scale Synthesis of **1**

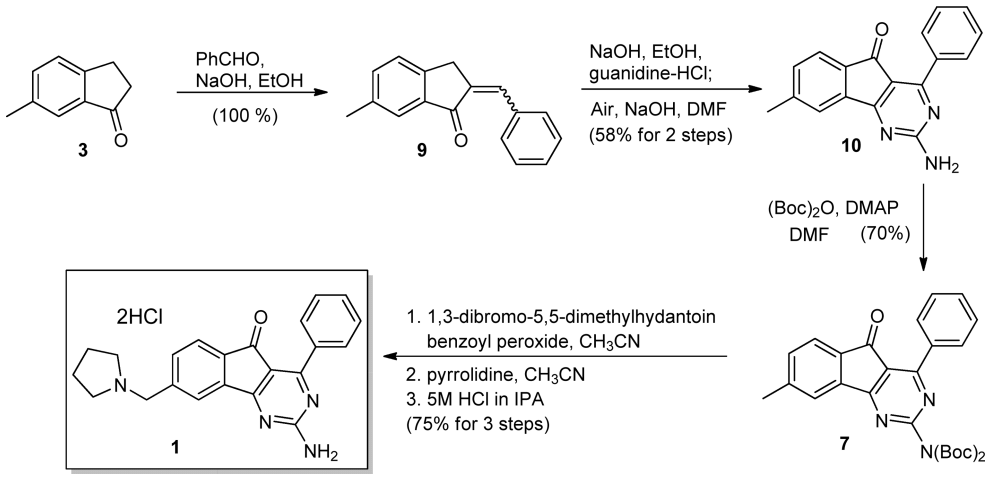


Table 1. Pharmacokinetic Data of Compound **1**

parameter	mouse (10 mg/kg, po)	rat (10 mg/kg, po)	monkey (10 mg/kg, po)
$t_{1/2}$ (h)	0.8	1.6	3.2
plasma C_{max} (nM)	655	1263	626
F (%)	100	64	39
brain C_{max} (nM)	ND	3552	ND

target compound **1**. This synthesis was completed in a total of seven steps starting from **3** and had an overall yield of 10.0%. This route was sufficient enough to prepare gram quantities of **1**; however, a better, more robust route needed to be designed to prepare **1** in large quantities.

The optimized large scale synthesis of **1** started with the same commercially available 6-methylindanone **3** which was condensed with benzaldehyde to afford the benzylidene **9** (Scheme 2).[20] The benzylidene **9** was reacted with guanidine and upon aromatization was oxidized to the corresponding ketone **10** using air in a one-pot reaction as described above.[26] The aminopyrimidine was protected with $(Boc)_2O$ to give the intermediate **7** that underwent radical initiated benzyl bromination similar to the discovery synthesis, but 1,3-dibromo-5,5-dimethylhydantoin proved to be a more robust brominating agent than NBS. The formed benzyl bromide was displaced in situ by pyrrolidine to give the di-Boc protected **1**, which was directly subjected to excess HCl to remove the Boc protecting groups and afford compound **1** as the di-HCl salt. The three steps were performed sequentially, without purification, to deliver the desired **1** as the di-HCl salt in 75% yield. This optimized synthesis removed three isolation/purification steps overall but more importantly removed the Suzuki-type coupling which used 20 mol % of $Pd(dppf)Cl_2$ which was tedious to remove down to acceptable levels. More than 300 g of **1** were prepared via this sequence in an overall yield of 30.5%.

The pharmacokinetics (PK) of **1** was determined in mouse, rat, and monkey (Table 1). In general, the compound had good oral bioavailability in all species ranging from 39% to 100% (Table 1). Plasma levels of **1** were modest in all species but had excellent exposure in rat brain with a brain/plasma (B/P) of ~3. The concentration of **1** found in rat brain (3552 nM) was 866-fold higher than the concentration needed to antagonize the A_{2A} receptor (K_i = 4.1 nM) and 208-fold higher than the needed concentration for the A_1 receptor (K_i = 17.0 nM). The oral plasma half-life was relatively low in rodent but was moderate in monkey.

In vivo efficacy of **1** was examined in a mouse model of neuroleptic-induced catalepsy.[21] Haloperidol, a neuroleptic medication that inhibits dopamine D_2 receptors,[29] was used to induce catalepsy. In the rodent, catalepsy is characterized as a loss of voluntary motion where limbs uncharacteristically remain in placed positions, which mimics the muscular stiffness seen in PD patients. Efficacy in this model is defined as reversal of catalepsy, i.e., moving from the placed positions (refer to the Experimental Section for a more detailed description of the mouse catalepsy model). This was the primary in vivo model that was used to evaluate compounds quickly by assessing oral and CNS activity. Also, the model typically required very little compound (5−10 mg), making it a robust tool with very fast turnaround. Compound **1** reversed haloperidol-induced catalepsy in mice in a dose-dependent fashion and had an ED_{50} of 0.17 mg/kg, po, while the minimum effective dose was shown to be 1 mg/kg, po (Figure 1). L-DOPA was used as the positive control.

As a point of reference, **2**, a selective A_{2A} receptor antagonist, was also run in the model in the exact fashion described above. Compound **2** showed positive results in a number of preclinical models of PD[30] and also showed positive clinical outcomes in PD patients.[31] The ED_{50} of **2** for inhibiting haloperidol-induced catalepsy in mice was 0.14 mg/kg, po, and the minimum effective dose was shown to be 1 mg/kg, po (Figure 1). The activity of **1** in the mouse catalepsy model was essentially the same as seen with the comparator **2**.

Another study was performed using the same model of neuroleptic-induced catalepsy in mice to examine the duration of action of **1**. The results in Figure 2 show that haloperidol induced catalepsy at each measurement period and that **1** was effective in reversing haloperidol-induced catalepsy at all measurement periods. Positive results were also observed for the A_{2A} receptor antagonist reference standard (**2**). These data suggest that in mice, the duration of action for in vivo efficacy of **1** is at least 4 h following oral administration at both 1 and 3 mg/kg. Extended measurement periods past 4 h were not examined nor were higher doses of compound **1**.

In vivo efficacy of **1** was examined in a rat model of neuroleptic-induced catalepsy.[22] This model was carried out analogously to that described for mice except that the maximum duration for rat was 180 s compared to 60 s for mice. Results in Figure 3 show that **1** was effective in reversing the haloperidol-induced catalepsy having an ED_{50} of 0.48 mg/kg,

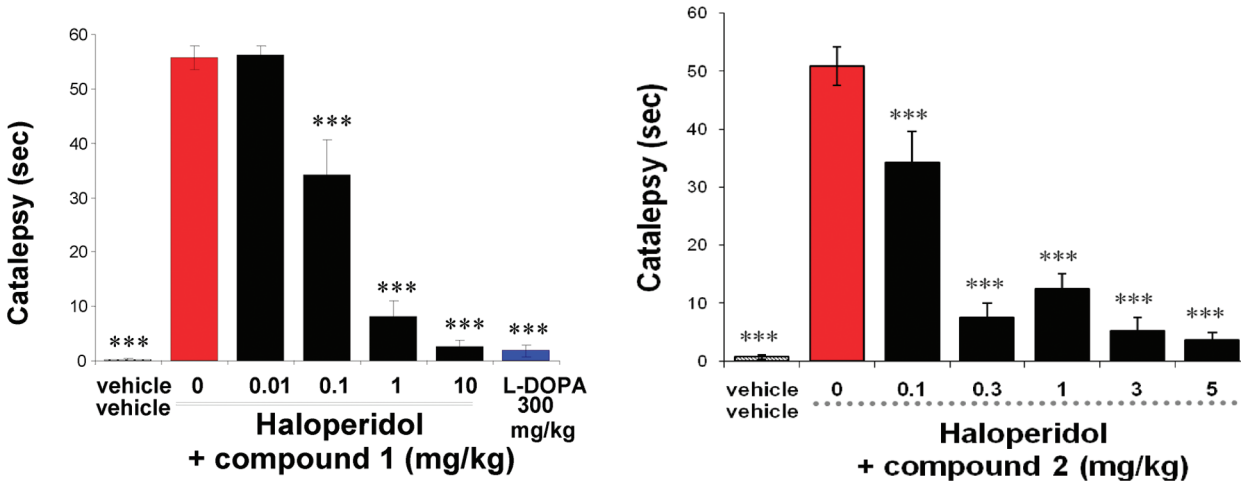


Figure 1. Reversal of neuroleptic-induced catalepsy in mice by compounds **1** and **2**. Left: Catalepsy was measured in haloperidol (1 mg/kg, sc) treated mice after oral administration of **1** (0.01, 0.10, 1.0, or 10.0 mg/kg, po). L-DOPA, the dopamine precursor and gold standard for treating PD, was used as the positive control for this model at 300 mg/kg, po. Right: Catalepsy was measured in haloperidol (1 mg/kg, sc) treated mice after oral administration of **2** (0.10, 1.0, 3.0, or 5.0 mg/kg, po). For both panels each value represents average (±SEM) time in cataleptic position of $n = 9-11$ mice per treatment group during a 60 s test session. Asterisks indicate significant differences compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

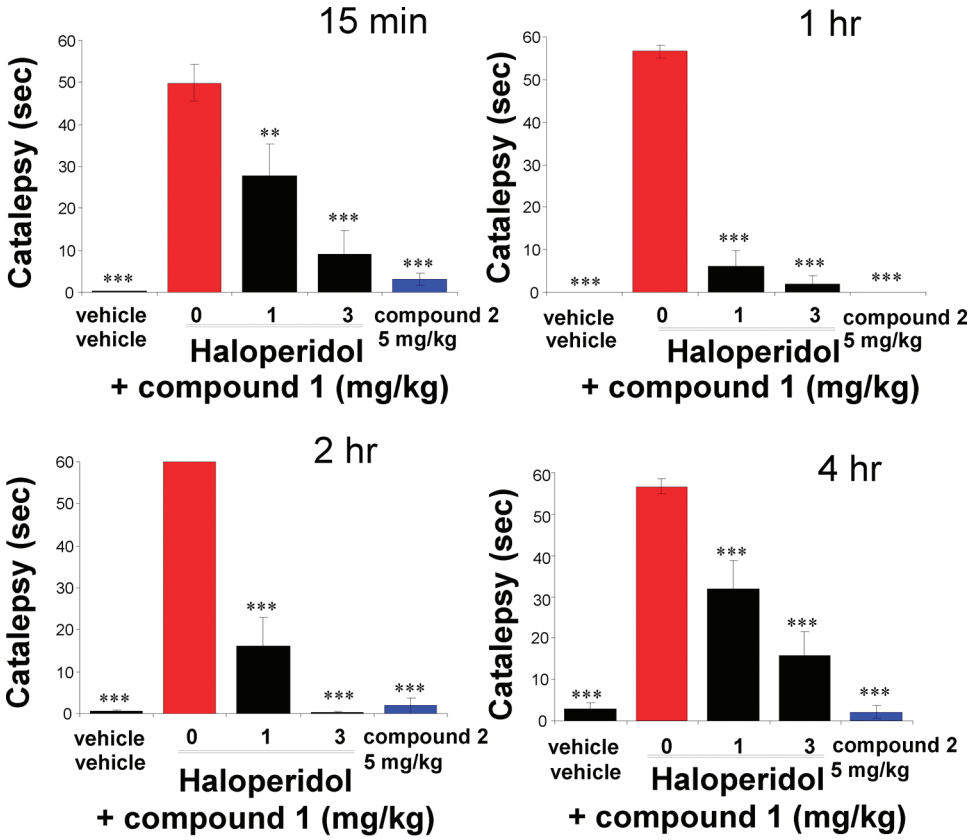


Figure 2. Duration of action of **1** in the mouse model of neuroleptic-induced catalepsy. Catalepsy time was measured in haloperidol (1 mg/kg, sc) treated mice after oral administration of **1** (0, 1, or 3 mg/kg), vehicle, or **2** (5 mg/kg). Catalepsy was measured repeatedly 15 min, 1 h, 2 h, and 4 h after oral dosing of the respective treatment groups. Each value represents average (±SEM) time in cataleptic position of $n = 12$ mice per treatment group during a 60 s test session. Asterisks indicate significant differences as compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

po, and a minimum effective dose of 1 mg/kg, po. As shown in mice, the reversal in rat was also dose-dependent.

A study was performed using the rat model of neuroleptic-induced catalepsy to examine the duration of action of **1**. The results in Figure 4 shows haloperidol induced catalepsy at each measurement period and that **1** was effective in reversing haloperidol-induced catalepsy at all measurement periods. Positive results were also observed for L-DOPA. These data suggest that in rats, the duration of action for in vivo efficacy of **1** is at least 4 h following oral administration.

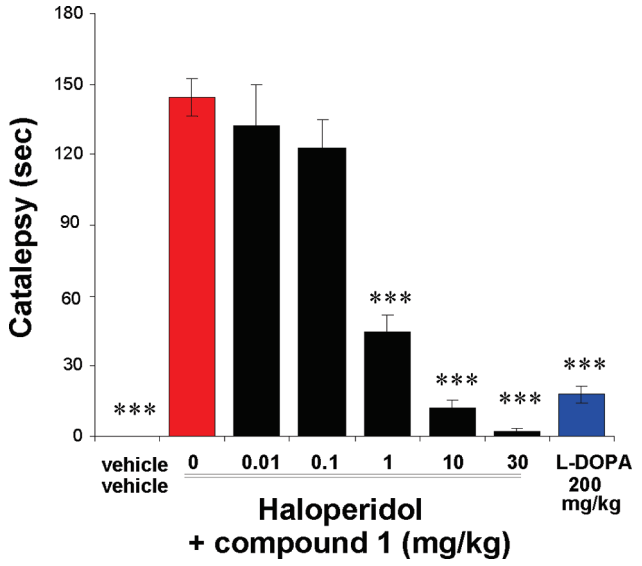


Figure 3. Reversal of neuroleptic-induced catalepsy in rats by compound **1**. Time to descend from the bar was measured in haloperidol (1 mg/kg, sc) treated Sprague−Dawley rats after oral administration of **1** (0.01, 0.10, 1.0, 10.0, or 30.0 mg/kg), L-DOPA coadministered with carbidopa (200:50 mg/kg), or vehicle. Each value represents average (±SEM) time in cataleptic position of n = 10−16 rats per treatment group during a 180 s test session. Asterisks indicate significant differences compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

To evaluate the potential antiparkinsonian properties of **1**, locomotor activity was studied in a mouse model of reserpine-induced akinesia.[23,32] Reserpine is an alkaloid that depletes monoamine by inhibiting their vesicular uptake, resulting in a dramatic reduction of spontaneous locomotor activity (akinesia). Efficacy was defined as reversal of reserpine-induced akinesia and was quantified as total distance traveled (cm) (refer to the Experimental Section for a more detailed description of the mouse reserpine model). Locomotion was studied 60 min after oral administration of **1** (0.10, 1.0, 10.0, or 30.0 mg/kg) or vehicle in mice that were pretreated with reserpine (0.6 mg/kg, sc) 18 h earlier. Reserpine produced a marked decrease in horizontal and vertical locomotor activity. As shown in Figure 5, reserpine-induced akinesia was reversed by **1** at 1, 10, and 30 mg/kg, po, and had a minimum effective dose of 1 mg/kg, po.

Compound **1** was studied in the 6-OHDA-lesion model of drug-induced rotation in rats.[24] In this model, the neurotoxin 6-OHDA (12 μg) is microinjected unilaterally in the medial forebrain bundle to produce a targeted degeneration of midbrain dopamine neurons in the pars compacta of the substantia nigra. 6-OHDA-induced neurotoxicity causes a dramatic deficit in dopamine neurons that is accompanied by denervation-induced supersensitivity of postsynaptic dopamine receptors in the striatum of the lesioned side. An imbalance in dopamine activity between the two sides of the brain causes asymmetry in motor behavior that can be enhanced by drug treatment. For example, drugs that stimulate

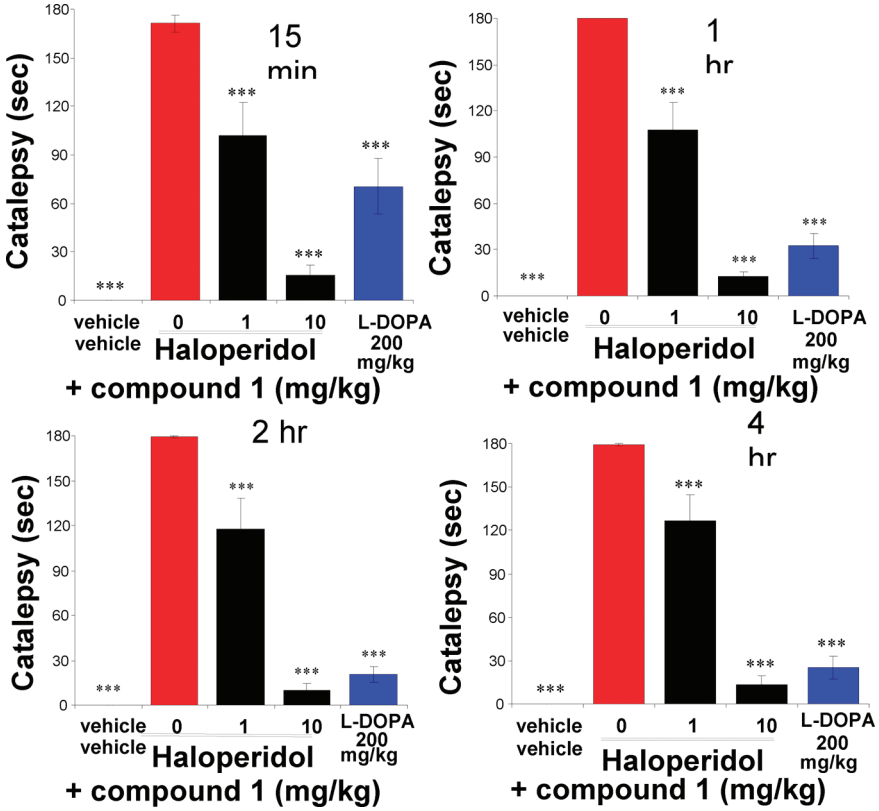


Figure 4. Duration of action of **1** in the rat model of neuroleptic-induced catalepsy. Time to descend from the bar was measured in haloperidol (1 mg/kg, sc) treated rats after oral administration of **1** (0, 1, or 10 mg/kg) or L-DOPA (200 mg/kg) coadministered with benserazide (50 mg/kg). Catalepsy was measured repeatedly 15 min, 1 h, 2 h, and 4 h after oral dosing of the respective treatment groups. Each value represents average (±SEM) time in cataleptic position of n = 10 rats per treatment group during a 180 s test session. Asterisks indicate significant differences compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

postsynaptic dopamine receptors produce an imbalance in dopamine signaling that favors the lesion side and induces rotation behavior (turning) toward the side opposite (contralateral to) the lesion side (refer to the Experimental Section for a more detailed description of the 6-OHDA-lesion model).[33]

One rotation count was defined as one 360° turn. Results showed that on its own, **1** produced a low level of behavioral activity that was less than the activity evoked by L-DOPA (Figure 6). More importantly, a dose-dependent, synergistic effect is realized when L-DOPA is administered to animals that were dosed previously with the adenosine receptor antagonist **1** with the minimum effective dose being 1 mg/kg, po. These results suggest that there must be some dopamine on board for an adenosine antagonist to be effective in treating PD, as there is little effect of **1** alone on the behavior of animals having minimal amounts dopamine neurons. It is known that approximately 80% of dopamine neurons are depleted before any PD symptom (i.e., motor dysfunction) is apparent.[34] It is therefore critical to identify PD patients at an earlier stage to maximize the benefit of adenosine receptor antagonists in treating PD. While this remains the ultimate challenge, significant efforts are being put forth to evaluate and treat PD patients to enhance quality of life.

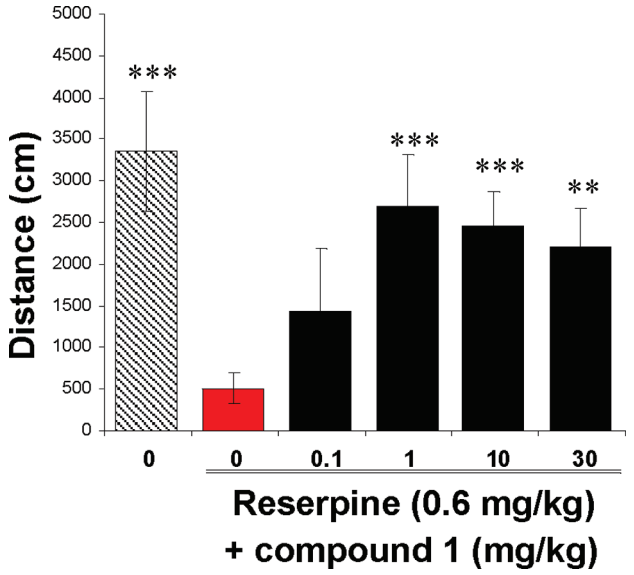


Figure 5. Reversal of resepine-induced akinesia in mice by compound **1**. Efficacy was defined as reversal of reserpine-induced akinesia and was quantified as total distance traveled (cm). Locomotion was studied 60 min after oral administration of **1** (0.10, 1.0, 10.0, or 30.0 mg/kg) or vehicle in mice that were pretreated with reserpine (0.6 mg/kg, sc) 18 h earlier. Each value represents the mean (±SEM) of the total distance traveled of $n = 10-12$ mice per treatment group during the 30 min measurement period in the behavioral test session. Asterisks indicate significant differences compared with the reserpine + vehicle control group (**, $P < 0.01$; ***, $P < 0.001$; Hochberg test of multiple comparisons).

The MPTP model in marmosets is perhaps the most predictive animal model of PD.[25] In this model, marmosets are administered a neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), that gives the animals nonreversible symptoms of PD. They possess similar motor disabilities and muscular rigidity seen in Parkinson's patients. The animals were administered **1** (0.3, 1.0, 10, and 50 mg/kg, po), L-DOPA as the positive control, or vehicle, and the Parkinson (motor) disability was scored. Doses of 10 and 50 mg/kg of **1** completely eliminated any motor disabilities in the marmosets for at least 6 h (the last time point scored), while a 1 mg/kg dose effectively eliminated the motor disabilities for ~3 h (Figure 7). L-DOPA (12 mg/kg po) showed suppression of the motor disabilities for less than 3 h before seeing significant motor dysfunction in the animals (refer to the Experimental Section for a more detailed description of the MPTP model).

In this model the dyskinesias, involuntary movements, in the animals were also scored. It is clearly seen that animals dosed with L-DOPA immediately show marked dyskinesias, while animals dosed with **1**, at all doses, showed no undesirable side effects. The results of this model showed that **1** could be an effective, stand alone treatment for PD without the major unwanted side effects.

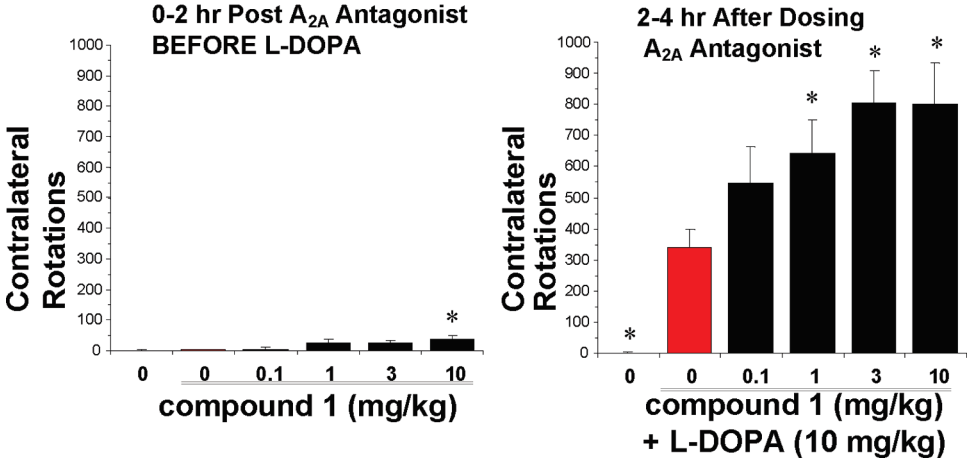


Figure 6. Compound **1** potentiates the effects of L-DOPA in the 6-OHDA-lesioned rat model of Parkinson's disease. Six weeks after administration of 6-OHDA the behavioral test was started by dosing rats with L-DOPA (10 mg/kg, po coadministered with carbidopa (2.5 mg/k, sc)) alone or with each dose of **1** (0.1, 1.0, 3.0, and 10 mg/kg, po). The effects of **1** alone were also studied in this model. The rotational behavior was determined as each animal was tethered to a high-resolution optical sensor connected to an automated computerized system that quantifies circular motion. One rotation count was defined as one 360° turn. Each value represents the mean (±SEM) of total contralateral rotations of $n = 16$ rats per treatment group during the 2 h before and after L-DOPA administration. Asterisks indicate significant differences compared with the L-DOPA + vehicle control treatment group (*, $P < 0.05$, Tukey's test of multiple comparisons).

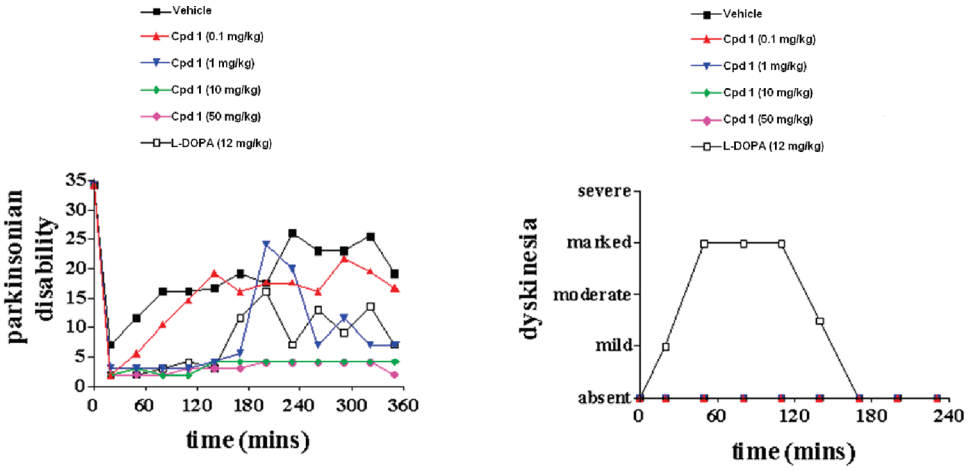


Figure 7. In the left panel the parkinsonian/motor disability was monitored and scored on MPTP-treated marmosets dosed with vehicle or L-DOPA (12 mg/kg, po) or with each dose of **1** (0.1, 1.0, 10, and 50 mg/kg, po) out to 6 h. In the right panel the dyskinesias were monitored and scored on MPTP-treated marmosets dosed with vehicle or L-DOPA (12 mg/kg, po) or with each dose of **1** (0.1, 1.0, 10, and 50 mg/kg, po) out to 4 h. A total of eight animals were used for each treatment group. Data derived from nonparametric data were analyzed with a nonparametric one-way repeated measures ANOVA (Friedman test) followed by Dunn's multiple comparison test.

In summary, we have shown that an optimized dual A_{2A}/A_1 receptor antagonist could be an effective treatment for PD. When administered orally, compound **1** was shown to have excellent efficacy across a number of animal models of PD including mouse and rat catalepsy, 6-OHDA lesion model in rats, and MPTP treated marmosets. Compound **1** was also able to demonstrate activity comparable to that seen with the comparator **2**.

Experimental Section

General Information. All proton and carbon nuclear magnetic resonance spectra were determined using a 400 MHz Bruker NMR with the appropriate internal standards. High resolution MS was performed on a JEOL Accutof JMS-T100 LC with a DART CE ionization source operating in the positive mode. Reagent grade chemicals and solvents were purchased from Aldrich, Oakwood, or TCI. All chromatographies were carried out on a CombiFlash system equipped with an automated fraction collector. All final compounds were purified to ≥95% purity as determined by Agilent 1100 series high performance liquid chromatography (HPLC) with UV detection at 254 nm using the following method: Supelcosil ABZ+PLUS, 3.3 cm × 2.1 cm, 11 min; 1.2 mL/min flow rate; 5−95% 0.1% TFA in CH_3CN/0.1% TFA in H_2O.

Small Scale (Discovery Synthesis) Preparation of 1. 2-Amino-4-phenyl-8-(pyrrolidin-1-ylmethyl)-5*H*-indeno[1,2-*d*]pyrimidin-5-one (1). Neat TFA (8 mL, 102.8 mmol) was added to a CH_2Cl_2 solution (24 mL) of **7** (1.94 g, 3.4 mmol), and the mixture was stirred at room temperature. After 2 h the solution was concentrated in vacuo and saturated aqueous $NaHCO_3$ was added to the material. The resulting suspension was sonicated and the precipitate was collected by filtration and washed with water. The collected solid was dried under high vacuum to afford 1.1 g (90%) of 2-amino-8-(bromomethyl)-4-phenyl-5*H*-indeno[1,2-*d*]-pyrimidin-5-one as a yellow solid that was used without further purification. t_R = 4.75 min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.03−8.12 (m, 2H), 7.89 (d, J = 0.98 Hz, 1H), 7.72 (d, J = 7.58 Hz, 1H), 7.46−7.62 (m, 4H), 5.88 (br s, 2H), 4.56 (s, 2H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 187.6, 176.1, 165.9, 164.5, 144.2, 140.6, 136.4, 135.2, 133.4, 131.4, 129.7, 128.1, 124.0, 122.0, 108.0, 32.1 ppm. HRMS, calcd for $C_{18}H_{12}BrN_3O$ 365.0164, found 366.0254 ($M^+ + H$).

Neat pyrrolidine (0.68 mL, 8.2 mmol) was added to a THF solution (20 mL) of 2-amino-8-(bromomethyl)-4-phenyl-5*H*-indeno[1,2-*d*]pyrimidin-5-one (1.5 g, 4.1 mmol), and the mixture was warmed to 40 °C. After 16 h the mixture was concentrated in vacuo, and the resulting material was dissolved in CH_2Cl_2 (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified via column chromatography to afford 1.1 g of **1** as a yellow solid. This material was dissolved in CH_2Cl_2 (60 mL) and added dropwise into 1 M HCl in ether (20 mL). The resulting yellow precipitate was collected by filtration and dried in vacuo to afford 1.2 g (75%) of **1** as the di-HCl salt. t_R = 3.07 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (br s, 1H), 8.12 (br s, 1H), 8.04 (s, 1H), 7.98−8.03 (m, 2H), 7.92 (dd, J = 1.22, 7.58 Hz, 1H), 7.74 (d, J = 7.58 Hz, 1H), 7.48−7.60 (m, 3H), 5.41 (br s, 2H), 4.52 (d, J = 5.62 Hz, 2H), 3.39 (d, J = 5.14 Hz, 2H), 3.09 (dd, J = 7.34, 10.76 Hz, 2H), 1.98−2.11 (m, 2H), 1.82−1.97 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 186.6, 175.3, 164.6, 139.8, 137.6, 136.7, 135.3, 135.2, 130.9, 129.4, 127.6, 123.2, 123.2, 123.1, 110.9, 57.7, 52.6, 22.5 ppm. HRMS, calcd for $C_{22}H_{20}N_4O$ 356.1637, found 357.1681 ($M^+ + H$).

2-(Bis(methylthio)methylene)-6-methyl-2,3-dihydro-1*H*-inden-1-one (4). A 1.0 M THF solution of NaHMDS (106 mL, 105.5 mmol) was added to a −78 °C THF solution (200 mL) of 6-methylindanone (**3**) (7.0 g, 47.9 mmol). After 30 min at −78 °C neat CS_2 (7.2 mL, 119.8 mmol) was added quickly in one portion to the cloudy solution. The mixture turned red, and the solution became homogeneous. The mixture was allowed to warm slowly to −20 °C without removal of the cold bath (~1.5 h). Then neat MeI (7.5 mL, 119.8 mmol) was added and the solution was allowed to warm to room temperature slowly overnight without removal of the cold bath. Water was added, and the layers were separated. The aqueous phase was extracted with EtOAc, and the combined organics were washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified via column chromatography to give 8.9 g of **4** as a yellow solid. t_R = 5.14 min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.61 (s, 1H), 7.31−7.40 (m, 2H), 3.81 (s, 2H), 2.57 (s, 3H), 2.52 (s, 3H), 2.41 (s, 3H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 189.3, 151.6, 144.5, 140.5, 137.3, 135.7, 134.9, 125.4, 124.0, 35.2, 21.2, 18.4, 17.9 ppm. HRMS, calcd for $C_{13}H_{14}OS_2$ 250.0486, found 251.0558 ($M^+ + H$).

2-Amino-8-methyl-4-(methylthio)-5*H*-indeno[1,2-*d*]pyrimidin-5-one (5). Solid **4** (8.9 g, 35.6 mmol) was added to a three-neck flask containing a DMF solution (100 mL) of guanidine−HCl (10.2 g, 106.8 mmol) and K_2CO_3 (14.8 g, 106.8 mmol), and the resulting mixture was heated to 100 °C while purging air into the reaction mixture. After 24 h the mixture was diluted with THF and EtOAc (200 mL each), and the mixture was washed with

water and brine, dried (Na_2SO_4), and dry-packed onto silica gel. Column chromatography afforded 4.8 g (53%) of **5** as a yellow solid. $t_R = 4.05$ min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.54−7.62 (m, 2H), 7.30 (d, J = 7.58 Hz, 1H), 5.56 (br s, 2H), 2.55 (s, 3H), 2.45 (s, 3H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 188.7, 174.3, 169.0, 163.8, 144.7, 140.1, 134.2, 132.9, 123.3, 122.2, 22.0, 11.3 ppm. HRMS, calcd for $C_{13}H_{11}N_3OS$ 257.0623, found 258.0728 ($M^+ + H$).

Bis-*tert*-butyl (8-Methyl-4-(methylthio)-5-oxo-5*H*-indeno[1,2-*d*]pyrimidin-2-yl)carbamate (6). Solid di-*tert*-butyl dicarbonate (12.2 g, 56.0 mmol) was added to a DMF solution (50 mL) of **5** (4.8 g, 18.7 mmol), 4-(dimethylamino)pyridine (228 mg, 1.8 mmol), and K_2CO_3 (12.9 g, 56.0 mol). After 18 h at room temperature the reaction mixture was diluted with EtOAc (300 mL) and the mixture was washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified via column chromatography to afford 6.1 g (72%) of **6** as a light yellow solid. t_R = 6.78 min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.58−7.68 (m, 2H), 7.34 (d, J = 7.58 Hz, 1H), 2.61 (s, 3H), 2.47 (s, 3H), 1.53 (s, 18H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 189.2, 173.7, 168.4, 160.0, 150.3, 146.1, 140.4, 133.5, 133.1, 123.9, 123.1, 118.6, 83.8, 27.9, 22.1, 14.1 ppm. HRMS, calcd for $C_{23}H_{27}N_3O_5S$ 457.1671, found 458.1776 ($M^+ + H$).

Bis-*tert*-butyl (8-Methyl-5-oxo-4-phenyl-5*H*-indeno[1,2-*d*]-pyrimidin-2-yl)carbamate (7). Solid Pd(dppf)Cl_2 (572 mg, 0.7 mmol) was added to a dioxane solution (60 mL) of **6** (1.6 g, 3.5 mmol), phenylboronic acid (854 mg, 7.0 mmol), and copper(I) thiophene-2-carboxylate (CuTC) (1.3 g, 7.0 mmol), and the resulting mixture was heated to 85 °C. After 20 h the mixture was diluted with EtOAc (100 mL) and filtered through medium porosity fritted funnel. The filtrate was washed 4 times with 10% aqueous NH_4OH, brine, dried (Na_2SO_4), and concentrated. The residue was purified via column chromatography to afford 1.25 g (74%) of **7** as a light yellow solid. t_R = 7.10 min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.18−8.26 (m, 2H), 7.75 (s, 1H), 7.68 (d, J = 7.58 Hz, 1H), 7.47−7.60 (m, 3H), 7.39 (d, J = 7.58 Hz, 1H), 2.51 (s, 3H), 1.52 (s, 18H) ppm. ^{13}C NMR (151 MHz, chloroform-*d*) δ 188.8, 176.4, 164.1, 160.7, 150.4, 146.6, 140.6, 134.5, 133.9, 133.1, 131.8, 130.3, 128.1, 124.2, 122.8, 118.7, 83.8, 27.9, 22.2 ppm. HRMS, calcd for $C_{28}H_{29}N_3O_5$ 487.2107, found 488.2160 ($M^+ + H$).

Bis-*tert*-butyl (8-(Bromomethyl)-5-oxo-4-phenyl-5*H*-indeno-[1,2-*d*]pyrimidin-2-yl)carbamate (8). Solid benzoyl peroxide (30 mg, 0.1 mmol) was added to a benzene solution (8 mL) of **6** (1.2 g, 2.5 mmol) and NBS (467 mg, 2.6 mmol), and the mixture was heated to reflux. After 16 h, the mixture was cooled to room temperature and diluted with EtOAc (50 mL). The mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified via column chromatography to afford 813 mg (57%) of **8** as a light yellow solid. An additional 342 mg (29%) of starting material **7** was also recovered. $t_R = 7.21$ min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.17−8.28 (m, 2H), 7.96 (d, J = 0.98 Hz, 1H), 7.77 (d, J = 7.58 Hz, 1H), 7.47−7.65 (m, 4H), 4.56 (s, 2H), 1.54 (s, 18H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 188.2, 175.8, 164.6, 160.9, 150.3, 145.4, 141.0, 135.1, 134.4, 133.9, 132.0, 130.3, 128.2, 124.7, 122.7, 118.4, 84.0, 31.8, 27.9 ppm. HRMS, calcd for $C_{28}H_{28}BrN_3O_5$ 565.1212, found 566.1311 ($M^+ + H$).

Large Scale Preparation of 1. 2-Amino-4-phenyl-8-(pyrrolidin-1-ylmethyl)-5*H*-indeno[1,2-*d*]pyrimidin-5-one Dihydrochloride (1). Solid 1,3-dibromo-5,5-dimethylhydantoin (89.8 g, 0.3 mol) was added to a 5 L four-neck flask equipped with a thermocouple controller, overhead mechanical stirrer, and a N_2 inlet/outlet adapter containing a CH_3CN solution of **7** (150 g, 0.3 mol) and benzoyl peroxide (4.5 g, 0.02 mol), and the mixture was refluxed. After 1 h at reflux the reaction mixture was cooled to room temperature, stored under nitrogen, and used directly in the next step without further purification.

Neat pyrrolidine (51 mL, 0.6 mol) was added to the same 5 L flask and stirred at room temperature. After 2 h, the reaction was complete, stored under nitrogen, and used directly in the next step without further purification.

A 5 M HCl solution in isopropyl alcohol (309 mL, 1.54 mol) was added to the same 5 L flask and stirred at room temperature. After 1 h the precipitate was collected by filtration, rinsed with CH_2Cl_2 (2 × 100 mL), and dried in vacuo at 60 °C for 18 h to afford 99.2 g (75%) of **1** as a di-HCl salt which was a yellow solid.

Bis-*tert*-butyl (8-Methy-5-oxo-4-phenyl-5*H*-indeno[1,2-*d*]pyrimidin-2-yl)carbamate (7). Solid di-*tert*-butyl dicarbonate (470 g, 2.1 mol) was added to a 12 L four-neck flask equipped with a thermocouple controller, overhead mechanical stirrer, and a N_2 inlet/outlet adapter containing a DMF solution of **10** (200 g, 0.7 mol), 4-(dimethylamino)pyridine (8.5 g, 0.07 mol), and K_2CO_3 (289 g, 2.1 mol). After 18 h at room temperature the reaction mixture was diluted with water and the resulting slurry was stirred for 2 h. The slurry was filtered, washed with water, and the collected solid was dried in vacuo to afford 314 g of crude **7**. The product **7** was dissolved in CH_2Cl_2 (1.5 L) and passed through silica gel (3 kg), eluting with CH_2Cl_2 (20 L). The collected filtrate was concentrated to afford 279 g (70%) of the title compound **7** as a white solid.

2-Benzylidene-6-methylindan-1-one (9). An aqueous solution (200 mL) of NaOH (65.7 g, 1.6 mol) was added to a 3 L three-neck flask equipped with an overhead mechanical stirrer charged with an EtOH solution (1.4 L) of 6-methylindan-1-one (**3**) (200 g, 1.4 mol) and benzaldehyde (153 mL, 1.5 mol). A white precipitate formed after the addition of NaOH was completed. The slurry became very thick, and stirring became difficult. So additional EtOH (1 L) was added. After 3 h the slurry was filtered, washed with cold EtOH and the collected precipitate was dried in vacuo to afford 321.0 g (100%) of the title compound **9** white solid. $t_R = 5.57$ min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.61−7.74 (m, 4H), 7.36−7.50 (m, 5H), 4.01 (s, 2H), 2.44 (s, 3H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 194.4, 147.0, 138.2, 137.6, 135.9, 135.5, 135.3, 133.7, 130.7, 130.7 129.6, 128.9, 128.9, 125.9, 124.5, 32.1, 21.2 ppm. HRMS, calcd for $C_{17}H_{14}O$ 234.1045, found 235.1134 ($M^+ + H$).

2-Amino-8-methyl-4-phenyl-5*H*-indeno[1,2-*d*]pyrimidin-5-one (10). Solid NaOH (187.8 g, 4.7 mol) was slowly added in four portions over 0.5 h to a 5 L four-neck flask equipped with a thermocouple controller, N_2 inlet/outlet adapter, and an overhead mechanical stirrer charged with an EtOH solution (2.7 L) of guanidine−HCl (453 g, 4.7 mol). After an additional 0.5 h, the slurry was filtered and the solid (NaCl) was washed with EtOH. The resulting filtrate, which contained the free base guanidine, was added in one portion to a 12 L four-neck flask equipped with a thermocouple controller, an overhead stirrer, and a condenser containing an EtOH suspension (1.1 L) of **9** (220 g, 0.94 mol). The heterogeneous solution was heated to 80 °C. After 30 min the reaction became homogeneous and was heated for an additional 18 h. The mixture was cooled to 0 °C, and the resulting precipitate was filtered. The collected solid was rinsed with cold EtOH and dried in vacuo to afford 185 g of a yellow solid that was carried on without further purification. The yellow solid (100 g) was added to a 5 L four-neck flask equipped with a thermocouple controller, overhead stirrer, and a N_2 inlet/outlet adapter. DMF (1.85 L) was added. The heterogeneous mixture was heated to 70 °C to form a homogeneous solution. Solid NaOH (43.6 g, 1.1 mol) was added, and the mixture was heated to 100 °C while air was purged into the reaction mixture. After 4 h the reaction mixture was cooled to room temperature, diluted with water and the resulting slurry was stirred. After 0.5 h the slurry was cooled to 0 °C and filtered. The collected solid was washed with water and dried in vacuo to afford 84.2 g (81%) of the title compound **10** as a red solid. $t_R = 4.25$ min. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.98−8.17 (m, 2H), 7.60−7.71 (m, 2H), 7.47−7.58 (m, 3H), 7.36 (d, J = 7.09 Hz, 1H), 5.77 (br s, 2H), 2.49 (s, 3H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*) δ 188.4, 176.7, 165.5, 164.5, 145.3, 140.3, 135.5, 134.2, 133.4, 131.1, 129.7, 128.0, 123.6, 121.9, 113.6, 22.1 ppm. HRMS, calcd for $C_{18}H_{13}N_3O$ 287.1059, found 288.1118 ($M^+ + H$).

Pharmacokinetic Studies. Compound **1** was administered to fasted Sprague−Dawley rats (male), BALB/c mice (male), and Cynomolus monkeys (male and female) by a single iv or oral administration. Compound **1** was formulated in 10% solutol for the iv injection. Compound **1** was formulated in 0.5% methylcellulose and dosed orally as a solution. The iv concentrations of **1** were 2 mg/kg for mice and rats and were 1 mg/kg for monkeys. The oral concentrations of **1** were 10 mg/kg for all species. Discrete blood samples collected from mice and rats were withdrawn from three to four animals per time point at selected intervals postdose via orbital sinus (rats) or cardiac (mice) puncture. Serial blood collections from monkeys were withdrawn by venipuncture. Plasma was obtained by centrifugation, processed by acetonitrile precipitation, and then analyzed by LC−MS/MS. The limit of quantitiation (LOQ) was 0.7−2 ng/mL. Noncompartmental analysis was performed on individual plasma concentrations using WinNonlin (version 4.0.4). For the tissue distribution studies a 10 mg/kg solution of **1** in 0.5% methylcellulose was administered orally to fasted Sprague−Dawley rats (male). At each time point four animlas were euthanized for collection of blood, plasma, and brain. Each tissue was weighed and homogenized in methanol. The methanolic extracts were evaporated and then reconstituted in mobile phase for analysis by LC−MS/MS. Blood and plasma was treated with acetonitrile and the supernatants analyzed for drug concentration by LC−MS/MS.

Mouse Catalepsy Study. Haloperidol, a neuroleptic medication that inhibits dopamine D_2 receptors, was used to induce catalepsy. In the rodent, catalepsy is characterized as a loss of voluntary motion where limbs uncharacteristically remain in placed positions. Catalepsy was measured in haloperidol (1 mg/kg, sc) treated mice (fasted, male BALB/c mice) after oral administration of **1** (0.01, 0.10, 1.0, or 10.0 mg/kg, po), L-DOPA (300 mg/kg, coadministered with carbidopa (75 mg/kg)), or vehicle. Animals were randomly assigned to treatment groups, and behavioral testing was performed blind to treatment. Control mice received the respective sc and po vehicles. Haloperidol was dissolved in 0.3% tartaric acid in 0.9% saline. L-DOPA was diluted in 0.5% methylcellulose and dosed as a suspension. Compound **1** was diluted in 0.5% methylcellulose and dosed as a solution. Compound **1** was administered orally 30 min after haloperidol. Behavioral testing was conducted 1 h after dosing of **1**. The behavioral test trial (maximum duration of 60 s) began by placing the forepaws of fasted, male BALB/c mice (18−23 g) on a horizontal bar elevated 3.5 cm above the bench. The cataleptic state was regarded as over and the trial ended when the animal came off the bar by either placing its forepaws on the bench or climbing onto the bar with all of its limbs. Each value represents average (±SEM) time in cataleptic position of n = 9−11 mice per treatment group during a 60 s test session. Asterisks indicate significant differences compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

A separate study was performed using the same model of neuroleptic-induced catalepsy to examine the duration of action of **1** in mice. Catalepsy time was measured in haloperidol (1 mg/kg, sc) treated mice (fasted, male BALB/c mice) after oral administration of **1** (0, 1, or 3 mg/kg), **2** (5 mg/kg), or vehicle. Compounds **1** and **2** were diluted in 0.5% methylcellulose. Catalepsy was measured repeatedly 15 min, 1 h, 2 h, and 4 h after oral dosing of the respective groups. Each value represents average (±SEM) time in cataleptic position of $n = 12$ mice per treatment group during a 60 s test session. Asterisks indicate significant differences compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

Rat Catalepsy Study. Haloperidol, a neuroleptic medication that inhibits dopamine D_2 receptors, was used to induce catalepsy. In the rodent, catalepsy is characterized as a loss of voluntary motion where limbs remain in placed positions. Time to descend from the bar was measured in haloperidol (1 mg/kg, sc) treated male Sprague−Dawley rats (fasted, 189−280 g) after oral administration of **1** (0.01, 0.10, 1.0, 10.0, or 30.0 mg/kg), L-DOPA coadministered with carbidopa (200:50 mg/kg), or vehicle. Animals were randomly assigned to treatment groups, and behavioral testing was performed blind to treatment. Control mice received the respective sc and po vehicles. Haloperidol was dissolved in 0.3% tartaric acid in 0.9% saline. L-DOPA/carbidopa were diluted in 0.5% methylcellulose and dosed as a suspension. Compound **1** was diluted in 0.5% methylcellulose and dosed as a solution. Compound **1** was administered orally 30 min after haloperidol. The behavioral test trial (maximum duration of 180 s) began by placing the forepaws of male Sprague−Dawley rats on a horizontal bar elevated 3.5 cm above the bench. The cataleptic state was regarded as over and the trial ended when the animal came off the bar by either placing its forepaws on the bench or climbing onto the bar with all of its limbs. Each value represents average (±SEM) time in cataleptic position of n = 10−16 rats per treatment group during a 180 s test session. Asterisks indicate significant differences compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

A separate study was performed using the same model of neuroleptic-induced catalepsy to examine the duration of action of **1** in rats. Catalepsy time was measured in haloperidol (1 mg/kg, sc) treated rats (fasted, male Sprague−Dawley rats) after oral administration of **1** (0, 1, or 10 mg/kg), L-DOPA coadministered with benserazide (200:50 mg/kg), or vehicle. L-DOPA was diluted in 0.5% methylcellulose and dosed as a suspension. Benserazide was diluted in saline and dosed as a solution. Compound **1** was diluted in 0.5% methylcellulose and dosed as a solution. Catalepsy was measured repeatedly 15 min, 1 h, 2 h, and 4 h after oral dosing of the respective groups. Each value represents average (±SEM) time in cataleptic position of $n = 10$ rats per treatment group during a 180 s test session. Asterisks indicate significant differences as compared with the haloperidol + vehicle control group (***, $P < 0.001$, Dunnett's test of multiple comparisons).

Reserpine Induced Akinesia Study. Reserpine is an alkaloid that depletes monoamines by inhibiting their vesicular uptake, resulting in a dramatic reduction of spontaneous locomotor activity (akinesia). Efficacy is defined as reversal of reserpine-induced akinesia. Locomotion was studied in open field activity boxes (L, 17.5 in.; W, 17.5 in.; H, 12 in.), each containing 20 equally spaced pairs of horizontal infrared photocell beams along one axis. Activity was measured automatically by a PC running the activity analysis software. One activity count corresponded to the consecutive interruption of two infrared beams placed 2.5 cm apart and 2 cm above the cage floor, and total distance traveled was quantified in cm. Locomotion was studied 60 min after oral administration of compound **1** (0.1, 1.0, 10, and 30 mg/kg, po) or vehicle in mice that were pretreated with reserpine (0.6 mg/kg, sc) 18 h earlier. Mice used were fasted, male CF-1 weighing 25−31 g at the start of the study. Animals were randomly assigned to treatment groups. Control mice received the respective sc and po vehicles. Reserpine was diluted in 0.5% acetic acid in distilled water and dosed sc. Compound **1** was diluted in 0.5% methylcellulose and dosed as a solution. Reserpine produced a marked decrease in horizontal and vertical locomotor activity. As shown in Figure 5, reserpine-induced akinesia was reversed by **1** at 1, 10, and 30 mg/kg, po. Each value represents the mean (±SEM) of the total distance traveled of n = 10−12 mice per treatment group during the 30 min measurement period in the behavioral test session. Asterisks indicate significant differences compared with the reserpine+vehicle control group (**, $P < 0.01$; ***, $P < 0.001$; Hochberg test of multiple comparisons).

Rat 6-OHDA Lesion Model of Drug-Induced Rotation. The neurotoxin 6-OHDA (12 μg) is microinjected unilaterally in the medial forebrain bundle to produce a targeted degeneration of

midbrain dopamine neurons in the pars compacta of the substantia nigra. Fasted, male Sprague−Dawley rats weighing 225−250 g at the time of surgery were used in this study. 6-OHDA-induced neurotoxicity is relatively selective for monoaminergic neurons because dopamine and noradrenergic transporters preferentially take it up. 6-OHDA accumulates in the cytosol of neurons and generates reactive oxygen species, which then inhibit mitochondrial respiratory enzymes leading to metabolic deficits and dopamine cell death. Degeneration of dopaminergic neurons in the injected side of the brain is accompanied by denervation-induced supersensitivity of postsynaptic dopamine receptors in the striatum of the lesioned side. An imbalance in dopamine activity between the two sides of the brain causes asymmetry in motor behavior that can be enhanced by drug treatment. For example, drugs that stimulate postsynaptic dopamine receptors produce an imbalance in dopamine signaling that favors the lesion side and induces rotation behavior (turning) toward the side opposite (contralateral to) the lesion side. Three weeks after administration of 6-OHDA, rats were challenged with drugs acting on the dopaminergic system and studied in a behavioral test chamber. Each animal was tethered to high-resolution optical sensor connected to an automated computerized system that quantifies circular motion. One rotation count was defined as one 360° turn. To test the effects of neuronal loss on drug responsiveness, motor asymmetry was examined in a screening test with subcutaneous administration of a postsynaptic dopamine agonist, apomorphine (0.05 mg/kg, sc). This test was repeated 1 week later and then followed 2 weeks later with a behavioral test to L-DOPA (10 mg/kg, po) coadministered with carbidopa (2.5 mg/kg, po). Animals showing an average response of at least 125 contralateral rotations in 1 h after apomorphine were included in further studies. Sixteen animals selected equidistant from the median L-DOPA response were used to test the effects of compound **1** on rotational behavior. To exclude a priming effect of L-DOPA, L-DOPA (10 mg/kg, po, coadministered with carbidopa (2.5 mg/kg, po)) was administered alone or with each dose of **1** (0.1, 1.0, 3.0, and 10.0 mg/kg, po) to all animals on separate testing days in a randomized order, with each rat serving as its own control. The effects of **1** alone were also studied in the model. This within-subjects repeated measure design was carried out with at least two nondrug days elapsing between drug testing sessions. Control rats received the respective po vehicles. Compound **1** was diluted in 0.5% methylcellulose and dosed as a solution. L-DOPA and carbidopa were diluted in 0.5% methylcellulose and coadministered as suspensions. Each behavioral test session began with a 30 min interval of acclimation in the test chamber, followed by administration of **1** or vehicle and measurement of behavior for 120 min. Thereafter, the behavioral effects of L-DOPA (coadministered with carbidopa) or vehicle were measured for 120 min. Each value represents the mean (±SEM) of total contralateral rotations of $n = 16$ rats per treatment group during the 2 h before and after L-DOPA administration. Asterisks indicate significant differences compared with the L-DOPA+vehicle control treatment group (∗, $P < 0.05$, Tukey's test of multiple comparisons).

MPTP-Marmosets. Marmosets (*Callithrix jacchus*) were rendered parkinsonian by subcutaneous injection of 2 mg/kg MPTP for 5 consecutive days. The marmosets were allowed to recover for a minimum of 10 weeks until their parkinsonism became stable. Stable dyskinesia was established by repeated L-DOPA therapy (12 mg/kg b.i.d. for 4 weeks). These animals have received other experimental treatments for Parkinson's disease, but a minimum period 2 weeks washout was allowed before commencing the study. There were eight marmosets available per treatment group for this study. All drugs were administered in the animal's home cage. The animals were immediately transferred to an observation cage (60 cm × 55 cm × 75 cm, with the perch 25 cm from floor of cage) for behavioral assessment. Parkinsonian disability was measured after oral administration of **1** (0.1, 1.0, 10.0, or 50.0 mg/kg), L-DOPA, or vehicle. Compound **1** was diluted in 0.5% methylcellulose and dosed as a solution. L-DOPA was suspended in 0.5% methylcellulose. Data derived from nonparametric data were analyzed with a nonparametric one-way repeated measures ANOVA (Friedman test) followed by Dunn's multiple comparison test.

A battery of behavioral tests was performed:

(1) activity, a quantitative assessment using computer-based activity monitors, was obtained every 5 min for the duration of the experiment.

(2) parkinsonian disability, nonparametric measures based on the following scales.

(a) range of movement score: 0 = no movement; 1 = movement of head on the floor of the cage; 2 = movement of limbs, but no locomotion, on the floor of the cage; 3 = movement of head or trunk on wall of cage or perch; 4 = movement of limbs, but no locomotion, on wall of cage or perch; 5 = walking around floor of cage or eating from hopper on floor; 6 = hopping on floor of cage; 7 = climbing onto wall of cage or perch; 8 = climbing up and down the walls of the cage or along perch; 9 = running, jumping, climbing between cage walls, perch, roof, using limbs through a wide range of motion and activity. The score given is the maximum achieved in each 10 min observation period.

(b) bradykinesia score: 0 = normal speed and initiation of movement; 1 = mild slowing of movement; 2 = moderate slowing, difficulty initiating and maintaining movement, marked freezing; 3 = akinetic, unable to move, with prolonged freezing episodes. The score given is representative of behavior over the observation period.

(c) postural abnormality score: 0 = normal, upright, holds head up, normal balance; 1= abnormal, crouched, face down, may lose balance. The score given is representative of behavior over the observation period.

(d) parkinsonian disability score: a combination of the mobility, bradykinesia and posture scores according to the formula [18 − (range of movement × 2) + (bradykinesia × 3) + (posture × 9)] to give a global parkinsonian disability rating.

(3) dyskinesiam nonparametric measures based on the following scale.

dyskinesia score: 0 = absent; 1 = mild, fleeting, present less than 30% of the observation period; 2 = moderate, not interfering with normal activity, present more than 30% of the observation period; 3 = marked, at times interfering with normal activity, present less than 70% of the observation period; 4 = severe, continuous, replacing normal activity, present more than 70% of the observation period.

Behavior was assessed for 6 h after drug administration. Behavioral test 1 (activity) was assessed every 5 min for 6 h after drug administration. Behavioral tests 2 and 3 (parkinsonian disability and dyskinesia, respectively) were assessed for 10 min every 30 min over the course of 6 h, by post hoc analysis of video recordings by an observer blinded to the treatment. The score given/achieved in each 10 min time period was presented as defined above.

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