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6-Alkoxyisoindolin-1-one based dopamine D_2 partial agonists as potential antipsychotics

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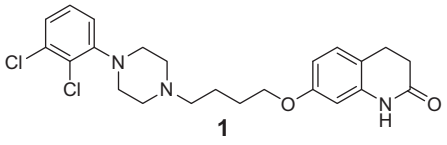
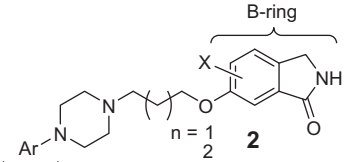
ABSTRACT

A series of 6-alkoxyisoindolin-1-ones with a magic shotgun pharmacological profile are presented as potential antipsychotics. The in vitro pharmacological profile includes D_2 partial agonism (30–55%), 5-HT_{1A} partial agonism (60–90%), and 5-HT_{2A} antagonism. Selected compounds in this series displayed good in vivo activity and potency.

Schizophrenia is a mental disorder that is characterized by positive symptoms such as delusions, hallucinations and disorganized speech/behavior. Negative symptoms include apathy, withdrawal, anhedonia, and impaired attention.[1] The atypical antipsychotics (risperidone, olanzapine, quetiapine and ziprasidone) are currently first-line therapeutics for schizophrenia. One of the newest antipsychotics to make its way to the market is aripiprazole (**1**, Abilify™), which was discovered by Otsuka[2] and introduced to the market by Bristol-Myers Squibb in 2002.[3] It has a different mechanism of action from the atypicals in that it is a D_2 partial agonist rather than an antagonist.[4] A partial agonist can uniquely moderate dopamine tone. It can act as an agonist on pre-synaptic autoreceptors, which have a high receptor reserve, and as an antagonist on D_2 post-synaptic receptors, where significant levels of endogenous dopamine exist and there is no receptor reserve.[5] The weak intrinsic agonist activity (IA) of 30% for aripiprazole prevents D_2 blockade from rising above 70%, which is still sufficient to achieve the 65% D_2 occupancy needed for a clinical response, but below the 80% D_2 occupancy where extrapyramidal side effects (EPS) are observed.[6] As well, this mechanism may provide for better subjective tolerability of treatment.[7] Consistent with this partial agonist mechanism, EPS was rarely observed with aripiprazole, even when striatal D_2 receptor occupancy values were above 90%.[8] Aripiprazole can be considered an atypical antipsychotic, since it is also an antagonist at 5-HT_{2A} receptors.[9] It is also a partial agonist at 5-HT_{1A} receptors which may provide some benefit against some of the negative symptoms of schizophrenia.[10] Clinical studies have demonstrated that aripiprazole is well tolerated and does not significantly induce EPS, weight gain, QT prolongation or increase plasma prolactin levels.[5]



1
aripiprazole (Abilify™)



2
isoindolin-1-one

In this Letter we present the in vitro and in vivo activity of 6-alkoxyisoindolin-1-ones based compound (**2**) as potential antipsychotics. Our target pharmacological profile was dopamine D_2 partial agonism (IA of 30–55%),[11] serotonin 5-HT_{1A} partial agonism (60–90%),[12] and serotonin 5-HT_{2A} antagonism.[13,14] We targeted a higher affinity at 5-HT_{2A} and 5-HT_{1A} versus D_2 to ensure maximal occupancy of several receptors when efficacious levels of D_2

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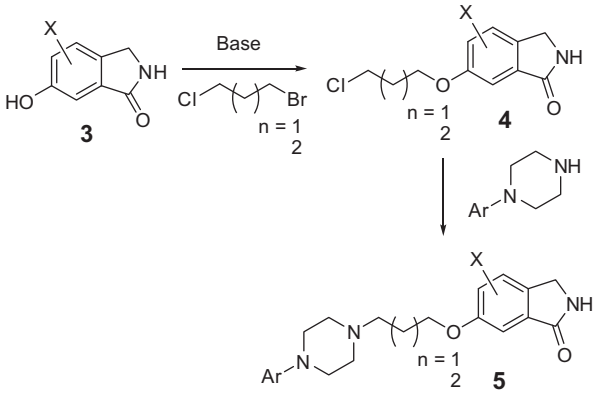


Scheme 1. General synthetic scheme.

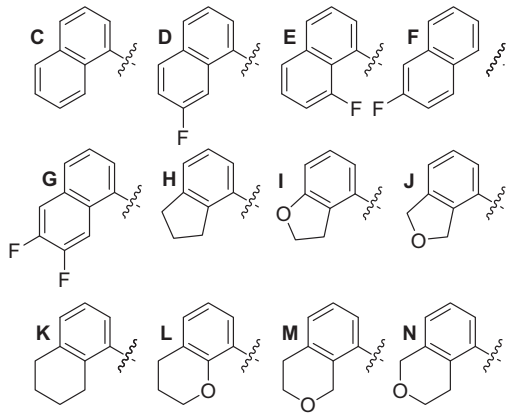
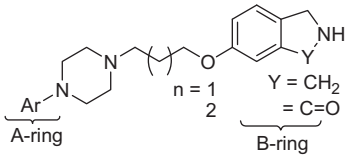


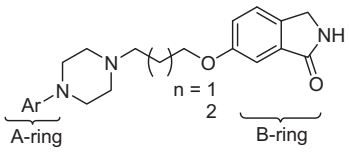
Figure 1. A-rings.

Table 1
In vitro activity of 5-alkoxyisoindoline and 6-alkoxyisoindolin-1-one based B-rings



Compd	A-ring	*n*	Y	D_2: K_i (nM)	5-HT_{2A}: K_i (nM)	5-HT_{1A} (1): K_i (nM)	5-HT_{1A} (2): K_i (nM)	D_2 % IA (TU)	D_2 % IA (FLPR)	5-HT_{1A} % IA (TU)	5-HT_{1A} % IA (FLPR)	DOF: K_i (nM)
1				4	4	7			29		43	598
7	C	1	CH_2	34	1.76	0.149	NT	27.8	NT	77.8	NT	779
8	C	2	CH_2	40.7	5.07	0.204	NT	35.3	NT	NT	NT	1560
9	D	1	CH_2	24.7	0.72	0.102	NT	41.3	NT	73.3	NT	931
10	D	2	CH_2	80.7	0.483	0.243	NT	58.7	NT	NT	NT	1680
11	C	1	C=O	16.3	14.9	0.0152	0.541	38.3	35.2	88.8	65.9	2810
12	C	2	C=O	7.39	4.95	0.151	2.22	55.3	32.1	66.7	NT	1850
13	D	1	C=O	27.8	3.37	0.0447	NT		59.1	NT	NT	3610
14	D	2	C=O	33.8	11.4	0.838	NT	21.3	53.9	NT	NT	NT

Table 2
In vitro activity of 6-alkoxyisoindolin-1-one based compounds



Compd	A-ring	*n*	D_2: K_i (nM)	5-HT_{2A}: K_i (nM)	5-HT_{1A} (1): K_i (nM)	5-HT_{1A} (2): K_i (nM)	D_2 % IA (TU)	D_2 % IA (FLPR)	DOF: K_i (nM)
15	C	1	16.3	14.9	0.0152	0.541	38.3	35.2	2810
12	C	2	7.39	4.95	0.151	2.22	55.3	32.1	1850
16	D	1	27.8	3.37	0.0447	NT	NT	59.1	3610
17	D	2	33.8	11.4	0.838	NT	21.3	53.9	NT
18	E	1	13.6	11.3	0.0141	NT	51.5	NT	1290
19	E	2	3.18	1.83	0.0504	0.951	50	38.2	2060
20	F	1	44.5	3.54	1.54	NT	NT	NT	439
21	F	2	58	9.53	13.4	NT	NT	NT	969
22	G	1	10.2	20.1	0.179	NT	NT	NT	907
23	G	2	8.23	7.91	1.3	NT	54.7	29.2	1860
24	H	1	0.302	3.16	NT	>4.04	NT	31.1	NT
25	H	2	0.0575	10.1	NT	0.74	NT	NT	NT
26	I	1	12.1	4.29	NT	1.46	NT	NT	NT
27	I	2	2.08	53.4	NT	1.45	NT	NT	NT
28	J	2	0.575	126	NT	6.88	NT	NT	NT
29	K	1	2.63	4.86	NT	0.269	NT	46.8	NT
30	K	2	1.54	3.35	NT	1.92	NT	NT	NT
31	L	1	4.47	25.3	NT	0.0501	NT	NT	NT
32	L	2	0.576	17.1	NT	0.863	NT	NT	NT
33	M	1	290	11.1	0.108	NT	NT	NT	NT
34	M	2	22.3	5.44	0.279	NT	39.2	NT	NT
35	N	2	11.5	11.2	1.99	NT	22.3	14.3	NT

occupancy were achieved. In vivo D_2 intrinsic activity (IA) was determined by blockade of the γ-butyrolactone (GBL)-induced increase in DOPA synthesis in mice.[15] Two primary behavioral models were used to evaluate compounds. Inhibition of spontaneous locomotor activity (sLMA) predicted efficacy for the positive symptoms of schizophrenia[16] and induction of catalepsy benchmarked the liability for extrapyramidal motor side-effects.[17] D_2 ex vivo binding was used to measure target occupancy for selected compounds in rat brain.

The general synthetic approach to isoindolin-1-one based compounds is shown in Scheme 1. The synthesis begins with alkylation of the hydroxyisoindolin-1-one core (**3**)[18] to install the 3- or 4-carbon linker followed by coupling to selected arylpiperazines to afford analogs **5**.[19] Figure 1 depicts the A-rings utilized to prepare the selected analogs.

Our initial investigations began with exploration of the 5-alkoxyisoindoline B-ring (Table 1, compounds **7**–**10**). We selected the napthyl-based A-rings (**C** and **D**) as we knew these conferred variety of unique 5-HT pharmacology to the template. The compounds in this series (**7**–**10**) had a desirable in vitro binding and functional profile at D_2, 5-HT_{2A} and 5-HT_{1A} but were weaker with respect to D_2 binding (Table 1).

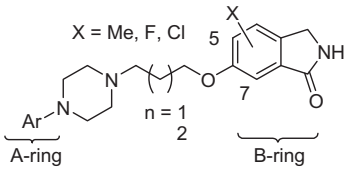
We next investigated the 6-alkoxyisoindolin-1-one B-ring (Table 1, Y = CH_2, compounds **11–14**). The initial compounds in this series had overall good in vitro binding and functional activity. These compounds also displayed favorable $\geqslant 1$ D_2/5-HT_{2A} ratio and had relatively low affinity in a dofetilide based assay to predict hERG liability. Encouraged by the initial **4** compounds in this series we chose to investigate a larger set of A-rings (Table 2). The most potent analogs at D_2 were those with 6,5-based A-rings (**H**, **I**, **J**) but the 6,6-based A-rings had a more desirable D_2/5-HT_{2A} ratio of >1 (Fig. 1).

In order to try and improve overall potency and/or the D_2/5-HT_{2A} and D_2/5-HT_{1A} ratios we next examined SAR of flouro, chloro and methyl substitution flanking the phenolic position (5- and 7-position, Table 3) of the isoindole B-ring. We chose to examine only a couple of different A-rings (**C**, **D**, **E**, and **F**) with these substituted B-rings. This provided compounds with generally good in vitro potency. Although, compounds with substitution on the 7-position of the isoindole ring generally had better affinity for 5-HT_{2A} and therefore provided a more attractive D_2/5-HT_{2A} ratio. Of the compounds tested in the D_2 functional assay, most had IA within our targeted range (IA of 30–55%). These substituted analogs, with an additional lipophilic atom on the isoindole ring, did not display profiles any better than the unsubstituted parent.

During or investigations we discovered that some of the compounds had significant activity as serotonin reuptake inhibitors (SRI, Table 4). We believe that addition of SRI activity to this class of compounds could have beneficial effects against the negative symptoms of schizophrenia.[20] A general trend is that compounds with a 3-carbon linker have better SRI potency than those with a 4-carbon linker.

The most optimally balanced profile in this series was achieved with compounds **12** and **19** and these were chosen for in vivo functional and behavioral studies (see Table 4). Both these compounds had good potency at D_2 and they both have D_2/5-HT_{2A} and D_2/5-HT_{1A} ratios >1. In addition, they both had desirable in vitro D_2 IA. The ability of these D_2 receptor partial agonists to block the GBL-induced increase in DOPA synthesis in the mouse brain was used to measure in vivo dopamine autoreceptor agonist intrinsic activity (Walters and Roth, 1976). This assay established that the D_2 IA of **12** and **19** is 47% and 60%, respectively (Table 5). Their in vivo D_2 receptor antagonist activity was assessed in the sLMA behavioral model, which is predictive of human antipsychotic efficacy. sLMA behavior is driven, at least in part, by endogenous dopaminergic tone.[11] Both **12** and **19** inhibit sLMA in rats in a dose-dependent

Table 3
In vitro activity of substituted 6-alkoxyisoindolin-1-one based B-rings



Compd	A-ring	*n*	B-ring	D_2: K_i (nM)	5-HT_{2A}: K_i (nM)	5-HT_{1A} (2): K_i (nM)	D_2 % IA (FLPR)
36	C	1	7-F	13.8	2.21	0.128	NT
37	C	2	7-F	22.3	1.08	3.62	NT
38	E	1	7-F	5.49	3.45	<0.140	52.6
39	E	2	7-F	35.2	0.568	2.09	NT
40	D	1	7-F	10.3	0.904	<0.205	53.6
41	D	2	7-F	5.75	2.1	4.56	40.3
42	F	1	7-F	8.59	5.41	3.04	29.9
43	F	2	7-F	15.8	3.07	5.01	NT
44	C	1	5-F	8.92	32.1	0.671	40.3
45	C	2	5-F	0.877	13	0.637	NT
46	E	1	5-F	9.54	1.19	<0.521	55.3
47	E	2	5-F	1.41	3.49	0.118	68
48	D	1	5-F	5.81	2.68	0.341	72
49	D	2	5-F	11.2	10	1.77	NT
50	F	1	5-F	1.86	38.5	0.88	NT
51	F	2	5-F	0.891	13.8	2.71	NT
52	C	1	7-Cl	10.6	2.06	0.786	NT
53	E	1	7-Cl	9.01	3.55	0.312	NT
54	E	2	7-Cl	9.86	1.13	4.25	NT
55	C	1	5-Cl	51.6	34.4	0.814	NT
56	C	2	5-Cl	9.31	24.7	2.34	NT
57	E	1	5-Cl	24.5	8.84	0.859	NT
58	E	2	5-Cl	5.91	25.3	1.68	NT
59	C	1	7-Me	6.81	9.43	0.615	NT
60	C	2	7-Me	13.1	1.26	3.22	NT
61	E	1	7-Me	13.6	16.1	2.13	NT
62	E	2	7-Me	3.03	3.85	2.61	NT
63	F	1	7-Me	1.7	12.5	1.08	NT
64	F	2	7-Me	9.44	14	17.5	NT
65	C	1	5-Me	21.8	55.9	0.67	NT
66	C	2	5-Me	2.46	36.8	0.803	NT
67	E	1	5-Me	16.6	65.7	1.44	NT
68	E	2	5-Me	2.34	55	3.61	NT
69	F	1	5-Me	3.81	52	0.517	NT
70	F	2	5-Me	0.812	29.5	1.98	NT

Table 4
SRI activity 6-alkoxyisoindolin-1-one based compounds

Compd	SERT SPA: K_i[a] (nM)	SERT: K_i[b] (nM)	Compd	SERT SPA: K_i[a] (nM)	SERT: Ki[b] (nM)
12	445	16.5	**53**	230	NT
19	NT	26	**54**	213	NT
36	NT	84.7	**55**	67.7	NT
37	NT	2520	**56**	96.3	NT
38	NT	160	**57**	20.2	NT
39	48.1	57.2	**58**	45.6	NT
40	447	1770	**59**	194	NT
41	NT	259	**60**	388	NT
42	24.7	37.4	**61**	437	NT
43	NT	2720	**62**	292	NT
44	NT	54.6	**63**	21.4	NT
45	NT	30.8	**64**	47	NT
46	NT	15.6	**65**	1040	NT
47	42.6	10.5	**66**	504	NT
48	419	13.6	**67**	174	NT
49	NT	110	**68**	118	NT
50	9.22	22.2	**69**	13.6	NT
51	13	9.1	**70**	8.81	NT
52	104	NT			

[a] 3H-citalopram binding to human serotonin transporter expressed in HEK 293 cells scintillation proximity assay (SPA).
[b] 3H-citalopram binding to human serotonin transporter expressed in HEK 293 cells.

Table 5
In vivo and ADME data of **1**, **12** and **19**[21]

Compd	1	12	19
In vivo D2 intrinsic activity @ 30 mg/kg, PO	43 ± 2	43 ± 2	60 ± 8
sLMA MED (mg/kg, PO)	3	1	1
c log *P*	4.4	4.74	4.89
HLM Clint, app (mL/min/kg)	31.7	23.7	39.6

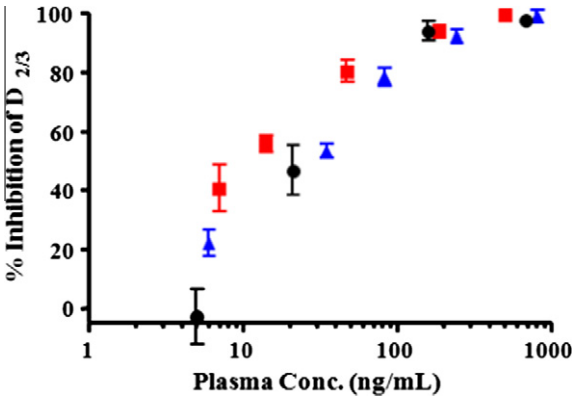


Figure 2. Effect of **1** (black circles), **12** (red squares) and **19** (blue triangles) on percent inhibition of ex vivo binding to D_2 receptors in rat striatum. Symbols represent mean percent inhibition of ex vivo [^{3}H]-raclopride binding in striatum + SEM. *N* = 4–5 rats per treatment group.

manner with a minimum effective dose (MED) of 1 mg/kg (Table 5). Preclinical measurement of catalepsy, which is an akinetic Parkinsonian-like state resulting from the prolonged blockade of dopaminergic neurotransmission, is useful for predicting the potential of antipsychotic drugs to produce EPS. When tested at 10 and 30 mg/kg, PO which is 10× and 30× the MED in sLMA, **19** did not satisfy the criteria for a cataleptic response. This compares to **1** which did cause catalepsy at 30 mg/kg.

An ex vivo D_2 binding assay with [^{3}H]-raclopride as a tracer was used to determine drug binding to the D_2 receptor in the striatum of rats (Fig. 2). The dosing regimen for these experiments was 0.3, 1.0, 3.0, 10.0, and 30 mg/kg, PO. It was important for our compounds to achieve high receptor occupancy with relatively low plasma concentrations. Compound **12** achieved 80% and 94% RO at plasma concentrations of 47 and 188 ng/mL, respectively, while compound **19** achieved 78.5% and 92.3% RO at plasma concentrations of 82 and 243 ng/mL, respectively. In comparison, aripiprazole (**1**) achieved 46% and 94% RO at plasma concentrations of 21 and 158 ng/mL, respectively. Both **12** and **19** had good brain/plasma ratios (2.49 and 1.81). The sLMA MED for was 1 mg/kg, PO for **12** and **19** which corresponded to D_2 RO of 55% and 53%, respectively. Compound **19** was tested in for catalepsy at a high dose of 30 mg/kg which correlated with 99% D_2 RO and 23× the plasma exposure needed to achieve the MED in sLMA. This compares to **1** which caused catalepsy at 30 mg/kg.

In conclusion, we have shown that a 6-alkoxyisoindolin-1-ones-based series of compounds display desirable in vitro pharmacological profiles with potent dopamine D_2 partial agonism (30–55%), serotonin 5-HT_{1A} partial agonism (60–90%),[22] and serotonin 5-HT_{2A} antagonism. Selected compounds in this series (**12** and **19**) displayed good in vivo activity (GBL and sLMA) and potency in an ex vivo binding D_2 binding assay. We believe the pharmacological profiles of **12** and **19**, especially the high affinity at 5-HT_{2A} and 5-HT_{1A} receptors compared to D_2, may provide a benefit in the treatment of negative symptoms, while maintaining antipsychotic activity against positive symptoms in patients with schizophrenia.

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11. Binding assay: [^{3}H]-Spiperone binding to a membrane preparation from CHO-hD2L cells; Functional assay: Intrinsic activity at the dopamine D_2 receptor was assesedusing [^{3}H]-thymidine uptake assay using CHO pro-5 cells and were compared to Quinpirole (full dopamine D_2 receptor agonist, 100% IA). Or, a D_2 receptors were engineered to artificially couple to calcium and functional agonist action were measured by incorporating calcium sensitive dyes into the cells and using a FLIPR to measure time-resolved changes in fluorescence.
12. Binding assay: 5-HT_{1A} (1): Competition binding of test compounds to h5-HT_{1A} receptors was conducted in membranes prepared from HeLa cells transfected with the cDNA for h5-HT_{1A} receptors using [^{3}H] 8-OH-DPAT as the receptor agonist. Or, 5-HT_{1A} (2): SPA bead/membrane based assay.
13. Binding assay: Schmidt, A. W.; Lebel, L. A.; Howard, H. R.; Zorn, S. H. *Eur. J. Pharmacol.* **2001**, *425*, 197.
14. For full assay details see Ref. 13.
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21. Data were normalized to 100% GBL effect and represent mean ± CI_{95}. Test compounds were administered PO 60 min prior to NSD/GBL, and then sacrificed 30 min later. Compounds were prepared using 0.5% methocel, 1% cremophor and 1% 1 N HCl. NSD = 3-hydroxybenzylhydrazine– an L-aromatic amino acid decarboxylase inhibitor; GBL = gamma butyrolactone.
22. Binding assay: Competition binding of test compounds to h5-HT_{1A} receptors was conducted in membranes prepared from HeLa cells transfected with the cDNA for h5-HT_{1A} receptors using [^{3}H] 8-OH-DPAT as the receptor agonist. Or, SPA bead/membrane based assay.