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Discovery of PF-00217830: Aryl piperazine napthyridinones as D_2 partial agonists for schizophrenia and bipolar disorder

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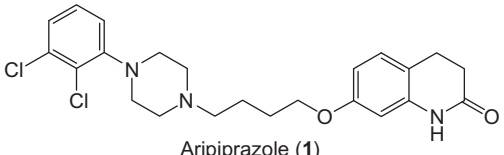
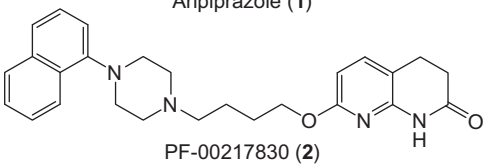
ABSTRACT

The synthesis and structure–activity relationship (SAR) of a novel series of aryl piperazine napthyridinone D_2 partial agonists is described. Our goal was to optimize the affinities for the D_2, 5-HT_{2A} and 5-HT_{1A} receptors, such that the D_2/5-HT_{2A} ratio was greater than 5 to ensure maximal occupancy of these receptors when the D_2 occupancy reached efficacious levels. This strategy led to identification of PF-00217830 (**2**) with robust inhibition of sLMA (MED = 0.3 mg/kg) and DOI-induced head twitches in rats (31% and 78% at 0.3 and 1 mg/kg) with no catalepsy observed at the highest dose tested (10 mg/kg).

Schizophrenia is a mental disorder that is characterized by positive symptoms such as delusions, hallucinations and disorganized speech/behavior and negative symptoms including apathy, withdrawal, lack of pleasure and impaired attention. The atypical antipsychotics (risperidone, olanzapine, quetiapine and ziprasidone) are all D_2 antagonists with relatively more potent blockade of 5-HT_{2A} receptors and they also possess a range of activities at other receptors.[1,2] Aripiprazole (**1**) was approved by the US Food and Drug Administration in 2002 as the first D_2 partial agonist for use in the treatment of psychiatric disorders.[3,4] 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.[5,6] Clinical studies have demonstrated that aripiprazole is well tolerated and does not significantly induce extrapyramidal side effects (EPS), weight gain, sedation, QT prolongation, prolactin elevation or tardive dyskinesia.[7,8] Atypical antipsychotics that are D_2 antagonists tend to cause EPS when the D_2 occupancy is above 80%.[9,10] In contrast, EPS was rarely observed with aripiprazole at clinically effective doses where approximately 90% of striatal D_2 receptors were occupied.[11–13] One hypothesis is that the partial agonist activity of aripiprazole (intrinsic activity (IA) of approximately 30%)[5] prevents the functional D_2 blockade from rising above 70%, which is above the 65% D_2 occupancy needed for a clinical response but below the 80% D_2 occupancy where EPS is observed.[10,14,15] Aripiprazole is also an antagonist at 5-HT_{2A} receptors and a partial agonist at 5-HT_{1A} receptors,[16] which may confer additional protection against the induction of EPS, as well as providing additional efficacy;[17] however the 5-HT_2 and 5-HT_{1A} receptor occupancy is considerably lower than that for the D_2 receptor.[12]



Aripiprazole (**1**)



PF-00217830 (**2**)

This Letter presents the discovery of PF-00217830 (**2**), a D_2 partial agonist (D_2PA) which advanced to phase 2 clinical studies as a treatment for schizophrenia.[18] Our target pharmacological profile was dopamine D_2 partial agonism (IA of 30–50%),[19] serotonin 5-HT_{1A} partial agonism (IA of 60–90%),[20] and serotonin 5-HT_{2A}

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antagonism.[21] We targeted a higher affinity at 5-HT_{2A} and 5-HT_{1A} to ensure maximal occupancy of these receptors when efficacious levels of D_2 occupancy were achieved. In vivo intrinsic activity (IA) was determined by blockade of the γ-butyrolactone (GBL)-induced increase in DOPA synthesis in mice.[22] Inhibition of spontaneous locomotor activity (sLMA) was used to evaluate compounds for efficacy against the positive symptoms of schizophrenia[23] and induction of catalepsy was used to benchmark the liability for EPS.[24] Changing the quinolinone core to a [1,8]-napthpyridinone ultimately resulted in the discovery of PF-00217830 (**2**), a compound that maintains a similar degree of D_2 partial agonism, but is a more potent antagonist and partial agonist at the 5-HT_{2A} and 5-HT_{1A} receptors, respectively. PF-00217830 was more potent than aripiprazole (**1**) in preclinical models predictive of antipsychotic activity (sLMA), whilst not inducing catalepsy at 30× the sLMA minimally effective dose (MED) suggesting the compound has a low likelihood of inducing EPS.

Our initial design focus was to investigate the effect of incorporating nitrogens in the B-ring while retaining the A-ring as dichloropiperazine (Table 1). Initially we made the carbon linked [1,8]-napthpyridinone **3**. We were encouraged that this compound had comparable D_2 affinity and IA as aripiprazole (**1**) with improved 5-HT_{2A} affinity. Unfortunately, **3** had very low oral bioavailability (%*F* = 6 in rat). We next made the oxygen linked version (**4**) and it had nearly the same activity at D_2, 5-HT_{2A} and 5-HT_{1A} as **3** with improved oral bioavailability (%*F* = 23 in rat). The corresponding dehydro-[1,8]-napthpyridinone analog **5** had a similar D_2 affinity as **4**, but the 5-HT_{2A} activity was diminished. We also investigated the activity of the [1,6]-napthpyridinone and the pyrido[2,3-*d*]pyrimidin-7(8*H*)-one B-rings (**6** and **7**). Analogs **6** and **7** had nearly the same D_2 receptor affinity, but had reduced affinity for 5-HT_{2A} and 5-HT_{1A} receptors compared to the corresponding [1,8]-napthpyridinone analog **4**. In addition, the D_2 IA of **6** and **7** (19% and 28%, respectively) was a little lower than what we were targeting. Therefore, based on the improved 5-HT_{2A} and 5-HT_{1A} activity and the higher D_2/5-HT_{2A} ratio of **4** compared to aripiprazole, we chose to further explore the SAR of the oxygen-linked [1,8]-napthpyridinone series.

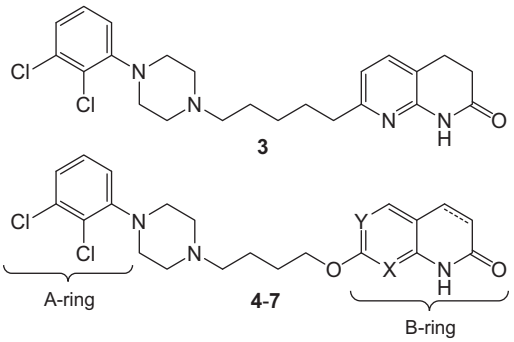
The general synthesis for the aryl piperazine [1,8]-napthyridinones (**15**) is shown in Scheme 1.[25] Treatment of 2,6-diaminopyridine with D,L-malic acid in concentrated H_2SO_4 provided aminonapthyridinone **8** in good yield. Diazotization of **8** in the presence of HF in pyridine gave fluoronapthyridinone **9** in excellent yield. Addition of 4-(benzyloxy)butan-1-ol to **9** using potassium *tert*-butoxide as base afforded **10**. The yield was improved by adding a catalytic amount of Bu_4NBr. Hydrogenation of **10** resulted in reduction to the dihydronaphthyridinone with concomitant removal of the benzyl group to give **13**. Oxidation of **13** with IBX followed by reductive amination of aldehyde **14a** with aryl piperazines provided the desired aryl piperazine dihydronaphthyridinones **15a**.

Alternatively, fluoronapthyridinone **9** was reacted with dry 1,4-butanediol and sodium hydride in NMP to give **12** directly or **9** was reacted with 4-(tetrahydro-2*H*-pyran-2-yloxy)butan-1-ol to give **11** followed by removal of the THP protecting group under mild conditions. Alcohol **12** was oxidized to aldehyde **14b** and subjected to various aryl piperazines under reductive amination conditions to give the aryl piperazine naphthyridinones **15b** in a similar manner as that described for the transformation of **13** to **15a**. Analogs with [1,6]-naphthyridinone and pyrido[2,3-*d*]pyrimidin-7(8*H*)-one B-rings were prepared in an analogous fashion.[25]

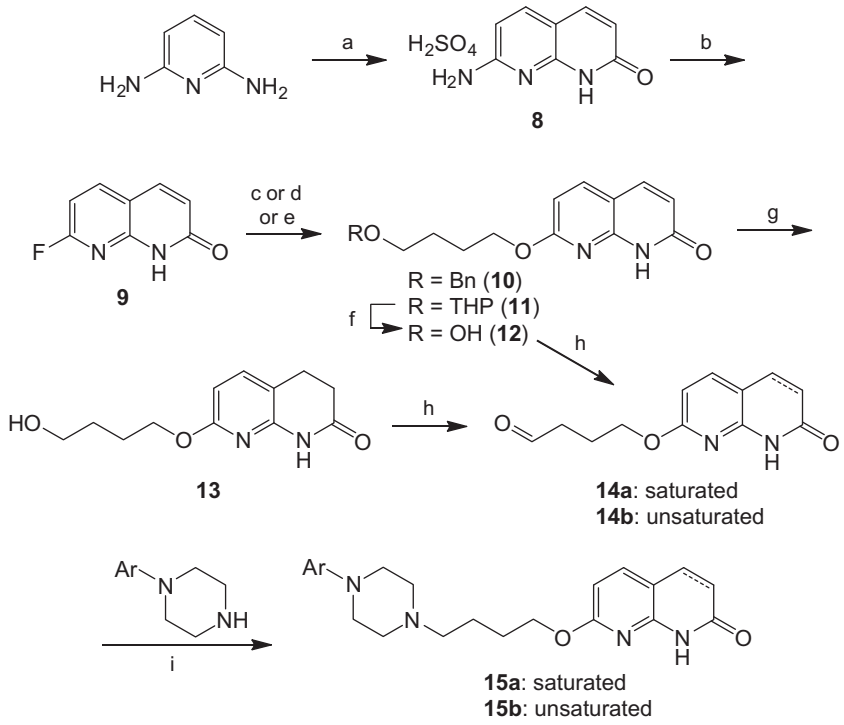
We next investigated the effect of substituents at the 2- and 3-position of the phenyl A-ring (Table 2). Substituents at both the 2- and 3-position of the phenyl ring were generally required for good 5-HT_{2A} activity. Replacing either of the chloro substituents of **4** with a methyl group led to compounds with improved 5-HT_{2A} activity and higher D_2/5-HT_{2A} ratios (**18** and **21**). The 2,3-dimethylphenylpiperazine compound **20** had a D_2/5-HT_{2A} ratio of 7, which was the highest of the substituted phenyl analogs that were evaluated. Analogs with only one substituent (i.e., **16** and **19**) or with a smaller fluoro substituent (i.e., **17** and **22**) had significantly inferior D_2/5-HT_{2A} ratios. A trifluoromethyl group was tolerated at the 2-position (**24**), but at the 3-position led to a lower D_2/5-HT_{2A} ratio and D_2 IA (**23**). Nearly all the analogs had D_2 intrinsic activities in the desired range (IA = 27–38%).

The potent D_2 and 5-HT_{2A} activity as well as the higher D_2/5-HT_{2A} ratio for the 2,3-dimethyl compound **20** led to the investigation of related bicyclic A rings (Table 3). The indane and tetrahydronaphthalene analogs **25** and **26** had excellent D_2/5-HT_{2A} ratios but the D_2 IA fell below our desired partial agonism range. Incorporating oxygen's in the benzylic positions of **26** resulted in similar D_2 and 5-HT_{2A} potency and D_2 IA, but the 5-HT_{1A} affinity improved.[26] Lastly we incorporated a naphthyl piperazine A-ring to give **2**. Compound **2** had subnanomolar affinity for the D_2 and

Table 1
SAR of B-rings

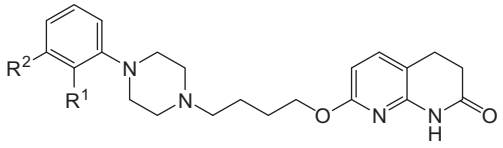


Compd	X	Y	bond	D_2(K_i, nM)	5-HT_{2A} (K_i, nM)	5-HT_{1A} (K_i, nM)	D_2%IA(TU)
1	CH	CH	Single	1.8	9.6	10.3	37
3				2.6	0.79	11.4	34
4	N	CH	Single	1.0	0.13	17.7	30
5	N	CH	Double	1.0	1.6	14.0	19
6	CH	N	Double	2.0	2.9	40.7	19
7	N	N	Double	1.0	3.2	52.7	28



Scheme 1. Reagents and conditions: (a) D,L-malic acid, H_2SO_4, 85%; (b) $NaNO_2$, HF-pyridine, 90%; (c) $BnO(CH_2)_4OH$, KO^{t}Bu, Bu_4NBr, THF, 90%; (d) $HO(CH_2)_4OH$, NaH, NMP, 68 °C, 62%; (e) $THPO(CH_2)_4OH$, KO^{t}Bu, NMP, 120 °C, 1 h, 89%; (f) HCl in dioxane, MeOH, 1 h, 94%; (g) H_2, Pd/C, MeOH, 95%; (h) IBX, EtOAc, 80 °C, 79–95%; (i) $NaBH(OAc)_3$, DCE, 50-80%.

Table 2
SAR of 2,3-substituted A-rings



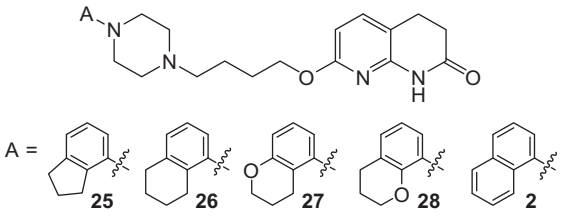
Compd	R^1	R^2	D_2 (K_i, nM)	5-HT_{2A} (K_i, nM)	5-HT_{1A} (K_i, nM)	D_2%IA (TU)
16	Cl	H	5.7	15.9	4.6	31
17	Cl	F	2.5	31.2	1.7	29
18	Cl	Me	1.0	0.50	13.8	29
19	Me	H	5.0	50.9	3.9	38
20	Me	Me	2.5	0.35	8.5	28
21	Me	Cl	3.0	1.5	8.4	35
22	F	Cl	1.0	10.4	1.2	31
23	Cl	CF_3	1.0	12.0	0.95	15
24	CF_3	Cl	3.0	1.4	3.5	33

5-HT_{2A} receptors and a D_2/5-HT_{2A} ratio of 6. In addition, the affinity at the 5-HT_{1A} receptor was improved compared to aripiprazole and the in vitro 5-HT_{1A} IA was 70–80% in a thymidine uptake functional assay, compared to an IA of 64% for aripiprazole. The D_2 intrinsic activity of **2** was similar to aripiprazole (**1**).

We next investigated the effects of fluorine substitution on the napthyl A-ring of **2** (Table 4).[27] Subtle changes with respect to the position of the fluorine substituent had large effects on the D_2 intrinsic activity, leading to compounds that range from antagonism (i.e. **29**) to higher intrinsic activity partial agonism (i.e., **35**). Incorporation of a fluorine at the 2-6 positions afforded compounds **29**–**33** with inferior profiles compared to **2**. They all had D_2/5-HT_{2A} ratios of <2 and D_2 intrinsic activities more in the range of an antagonist (IA = 3–19%). However, addition of a fluorine substituent at the 7- or 8-position of the naphthyl ring afforded compounds **34** and **35** with good D_2 affinity and IA, and excellent 5-HT_{2A} and 5-HT_{1A} potency. In addition, compound **34** had a D_2/5-HT_{2A} ratio >4. Notably, incorporation of a fluorine at the 8-position afforded a compound (**35**) with significantly higher D_2 IA compared with aripiprazole (51% vs 36%). Therefore, compounds **2** and **34** met our target profile for a D_2 partial agonist similar to **1** with enhanced serotonergic pharmacology and the in vivo characterization of **2** (known as PF-00217830) is discussed below.

The ability of PF-00217830 (**2**) to block the GBL-induced increase in DOPA synthesis in the mouse brain was used to measure in vivo dopamine autoreceptor agonist intrinsic activity.[22] The level of partial agonism is determined by the degree of maximum reversal of the GBL effect reflecting the IA of the compound (Table 5).[28] The in vivo intrinsic activity of **2** (GBL) was a little higher than the in vitro intrinsic activity (D_2 TU), but **2** was still

Table 3
SAR of bicyclic A-rings



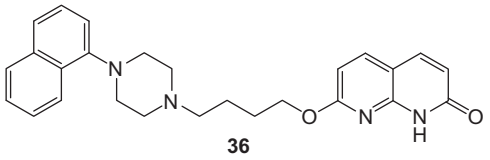
Compd	D_2 (K_i, nM)	5-HT_{2A} (K_i, nM)	5-HT_{1A} (K_i, nM)	D_2%IA (TU)
25	1.0	0.16	6.3	18
26	1.4	0.09	28	21
27	1.0	1.3	0.78	18
28	0.71	0.19	1.1	14
2	0.81	0.14	3.7	38

determined to be a partial agonist in our desired range (IA of 30–50%). The in vivo D_2 receptor antagonist activity was initially 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.[23] Compound **2** inhibited sLMA in rats in a dose-dependent manner with a minimum effective dose (MED) of 0.3 mg/kg po (Table 5). Preclinical measurement of catalepsy, an akinetic state resulting from the blockade of dopaminergic neurotransmission, is a useful model for predicting the propensity of antipsychotics to produce EPS.[29] When tested at 10 mg/kg, po which is 33× the MED in sLMA, **2** did not show a cataleptic response. In rat ex vivo D_2 receptor occupancy studies using inhibition of [^{3}H]raclopride binding, **2** (10 mg/kg, po) produced >90% occupancy, further supporting the probability that **2** would have a low likelihood of inducing EPS.[30] In addition, the 5-HT_{2A} receptor antagonism was assessed in vivo using a DOI-induced head twitch assay. 5-HT_{2A} behavioral effects were induced by 2,5-dimethoxy-4-iodoamphetamine (DOI), a non-subtype-selective 5-HT_2 agonist, that produces stereotypic head twitches, which are blocked by selective 5-HT_{2A} receptor antagonists.[31] **2** reduced the number of DOI-induced head twitches in rats by 31% and 78%, respectively, at doses of 0.3 and 1 mg/kg po (Table 5). In the course of these studies, we found that **36** was an active metabolite of **2**. Therefore, **36** was profiled in vitro[32] and in vivo and it was found to have a similar pharmacological profile as **2** (Table 5).

Table 5
In vivo pharmacology summary for aripiprazole (**1**), PF-00217830 (**2**) and its active metabolite **36**

Assay	**1**	**2**	**36**
% Blockade of GBL (po)	43%	46%	45%(sc)
sLMA (MED, mg/kg, po)	3.0	0.3	1.0
DOI-induced head twitch[a] (% inhibition)	38%	78%	ND
Catalepsy[b] (MED, mg/kg, po)	30	>10	>30
Ratio of catalepsy MED to sLMA MED	10	>30	>30

[a] % Inhibition of DOI-induced head twitches in rats @ 3× sLMA MED.
[b] Measure of EPS liability.

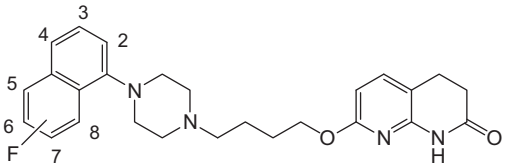


36

The pharmacokinetic profile of PF-00217830 (**2**) was examined in rat and monkey (Table 6).[33] Following iv administration of **2**, mean plasma clearance values were 14.9 and 5.8 mL/min/kg in rat and monkey, respectively, indicating low to moderate clearance in these species. The mean volume of distribution at steady state (V_{dss}) values for rat (1.13 L/kg) and monkey (2.62 L/kg) were greater than total body water (approximately 0.6–0.7 L/kg), indicating that **2** distributes to tissues. The mean absolute oral bioavailability in rats and monkeys was 19.2% and 20.5%, respectively. In toxicology studies with rats and monkeys, C_{max} and AUC values for **2** increased in a dose-related manner over the dose range evaluated. PF-00217830 (**2**) was highly protein bound in plasma with an unbound fraction of 1.79%, 0.14%, 0.62% and 0.34% in mouse, rat, monkey, and human plasma, respectively. PF-00217830 (**2**) is not a P-gp substrate and crosses the blood-brain barrier in rats (B/P $AUC_{(0-7h)}$ ratio = 0.7).

In vitro metabolism of PF-00217830 (**2**) is consistent across nonclinical species and humans. All metabolites observed in human in vitro incubations are present in one or more of the evaluated nonclinical species. In vitro, CYP3A4 and 2D6 play a role in the metabolism of **2**. In vitro studies using pooled human liver microsomes demonstrated low potential of **2** to inhibit activities

Table 4
SAR of fluoro substituted napthyl A-rings



Compd	F-position	D_2 (K_i, nM)	5-HT_{2A} (K_i, nM)	5-HT_{1A} (K_i, nM)	D_2%IA (TU)
29	2	1.6	6.7	0.84	3
30	3	1.1	1.9	3.3	14
31	4	2.0	11.7	2.0	10
32	5	6.0	3.3	3.0	17
33	6	1.0	3.5	1.2	19
34	7	1.2	0.28	1.1	36
35	8	1.7	0.67	0.77	51

Table 6
Plasma pharmacokinetic data following administration of PF-00217830 (**2**) (iv dose of 1 mg/kg and po dose of 3 mg/kg) in male rats and monkeys ($n = 3$)

Species	C_{max} (ng/mL)	t_{max} (h)	CL (mL/min/kg)	V_{dss} (L/kg)	iv $t_{1/2}$ (h)	F (%)
rat	202	1.2	14.9	1.1	6.8	19.2
monkey	96	5.0	5.8	2.6	13.6	20.5

of CYP1A2, 2C9, 2C19, 2D6, and 3A4 based on IC_{50} values relative to the predicted efficacious concentration (75.6 ng/mL) in humans.

In summary, PF-00217830 (**2**) met our laboratory objectives including an increased affinity for D_2, 5-HT_{2A} and 5-HT_{1A} receptors relative to **1**, D_2/5-HT_{2A} > 5, D_2 IA = 38–46% and 5-HT_{1A} IA = 70–80%. Furthermore, **2** inhibited sLMA in rats with a minimum effective dose of 0.3 mg/kg and it reduced the number of DOI-induced head twitches in rats by 31% and 78% (0.3 and 1 mg/kg, po) with no catalepsy observed at the highest dose tested (10 mg/kg, po).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.059. These data include MOL files and InChiKeys of the most important compounds described in this article.

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19. 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 assessed using [^{3}H]-thymidine uptake assay using CHO pro-5 cells and were compared to Quinpirole (full dopamine D_2 receptor agonist, 100% IA).
20. 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.
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22. In vivo D_2 intrinsic activity (IA) was determined by blockade of the γ-butyrolactone (GBL)-induced increase in DOPA synthesis in mice. Whetzel, S. Z.; Shih, Y. H.; Georgic, L. M.; Akunne, H. C.; Pugsley, T. A. *Neurochem* **1997**, *69*, 2363. In all in vivo studies, the practices and procedures were conducted in accordance with the NIH Guidelines for Care and Use of Laboratory Animals.
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28. 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% 1N HCl. NSD = 3-hydroxybenzylhydrazine, an L-aromatic amino acid decarboxylase inhibitor; GBL = γ-butyrolactone.
29. Catalepsy is measured by placing animals in an unusual posture (grasping a bar above an elevated surface) and measuring the latency to move from the abnormal position. Vehicle treated controls spend on the average 7–8 s in this position. Compounds are judged as producing catalepsy when the majority of animals spend more than 20 s in the unusual postural position.[22]
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32. In vitro binding K_is for **36**: D_2 0.4 nM (IA = 44%), 5-HT_{2A} 0.1 nM, 5-HT_{1A} 0.2 nM.
33. Plasma pharmacokinetic data following administration of aripiprazole (**1**) (iv dose of 1 mg/kg and po dose of 20 mg/kg in male rats): C_{max} = 289 ng/mL, t_{max} = 1.5 h, %F = 16.