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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF THE ENANTIOMERS OF THE DOPAMINE AUTORECEPTOR AGONIST PD 135385

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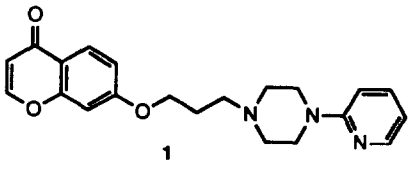
The clinical efficacy of traditional antipsychotic compounds is directly related to their ability to block brain dopamine (DA) D_2 receptors.[1] Unfortunately, their extrapyramidal side effects are also intimately associated with DA receptor blockade.[2] Selective activation of presynaptic DA receptors (DA autoreceptors) represents an alternative therapeutic strategy for the modulation of DA neuronal function.[3] DA autoreceptors serve a feed-back function, and they exert an inhibitory influence on DA neuronal firing, as well as DA synthesis and release.[4] Activation of DA autoreceptors without concurrent stimulation of post-synaptic DA receptors requires the development of selective DA autoreceptor agonists.[5] Recent developments in the molecular biology of DA receptors suggest that the difference between DA autoreceptors and postsynaptic receptors is one of sensitivity rather than one of structural diversity.[6] DA autoreceptors are classified pharmacologically as DA D_2 receptors,[7] yet show a greater sensitivity to DA agonists than do postsynaptic DA D_2 receptors.[8] This sensitivity difference may be due to the greater DA receptor reserve at presynaptic versus postsynaptic sites.[9] It has become increasingly clear that a compound must be a partial D_2 agonist in order to behave as a selective DA autoreceptor agonist *in vivo*. The precise level of efficacy required falls within a very narrow range. Sufficient intrinsic activity must exist to adequately stimulate DA autoreceptors, but DA agonists that possess too much intrinsic efficacy may stimulate DA postsynaptic receptors, possibly resulting in exacerbation of the psychosis. Compounds such as PD 119819 (**1**)[10] and (±)-PD 128483 (**2**)[11] have been described that meet these requirements. This paper describes the synthesis and preliminary biological characterization of a novel compound, PD 135385 (**18**), and its enantiomers, which appear to possess an optimal profile of partial DA agonism.



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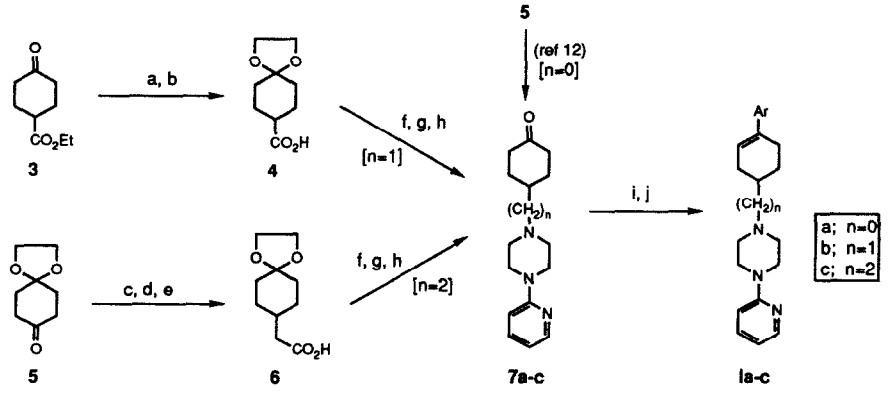
Our earlier work with DA autoreceptor agonists led us to postulate an arylpiperazine binding site within the D_2 receptor.[10] Several series of arylpiperazine-containing compounds have been identified that bind to D_2 receptors and range from D_2 antagonists to efficacious D_2 agonists.[10,12] As a continuation of this work, a series of compounds of generic structure **I** was targeted for synthesis. The (2-pyridyl)piperazine moiety was chosen as the optimal arylpiperazine based on our earlier work.[10] Representative carbocyclic and heterocyclic examples are shown in Table I (Ar= phenyl, pyridyl, thienyl). Compounds with a side chain ranging in size from n=0 to n=2 were synthesized (Table I).

Chemistry

The synthesis of the target compounds is outlined in Schemes 1 and 2. Reaction of substituted cyclohexanone **7a**[13] with aromatic organometallic reagents, followed by acid-catalyzed dehydration, provided compounds **Ia**.

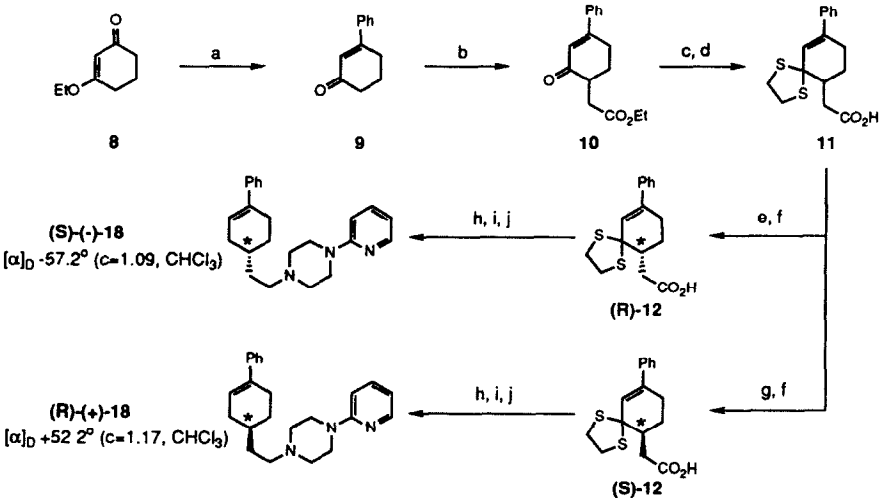
The longer analogues were prepared by conversion of either carboxylic acid **4** or **6** to the corresponding (2-pyridyl)piperazine amides, followed by amide reduction and hydrolysis of the ketal group to give the desired substituted cyclohexanones **7b** and **7c**. These compounds were then reacted with the appropriate aryl-lithium or aryl-magnesium reagents, followed by acid-catalized dehydration to yield the target compounds **Ib** and **Ic**.

Scheme 1. Synthesis of Ia-c



[a] $HOCH_2CH_2OH$, benzene, *p*-TsOH; [b] NaOH, EtOH, H_2O; [c] $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 0 °C; [d] H_2, 5% Pd/C, EtOH; [e] NaOH, EtOH, H_2O; [f] i: $^tBuOCOCl$, Et_3N, CH_2Cl_2, 0 °C, ii: 1-(2-pyridyl)piperazine; [g] $LiAlH_4$, $AlCl_3$, THF, Et_2O; [h] 10% HCl, acetone; [i] ArLi or ArMgBr, THF, -78 °C; [j] *p*-TsOH, toluene, ($-H_2O$).

Scheme 2. Resolution of PD 135385



[a] i: PhLi, Et_2O, 0 °C; ii: HCl; [b] i: LDA, THF, -78 °C; ii: $BrCH_2CO_2Et$; [c] $HSCH_2CH_2SH$, BF_3 Et_2O, CH_2Cl_2, -20 °C, [d] NaOH, EtOH, H_2O, reflux; [e] (R)-(-)-$PhCH(NH_2)CH_2OH$, EtOH, Et_2O; [f] Recrystallize from EtOH (3x); [g] (S)-(+)-$PhCH(NH_2)CH_2OH$, EtOH, Et_2O; [h] i: $^tBuOCOCl$, Et_3N, CH_2Cl_2, 0 °C; ii: 1-(2-pyridyl)piperazine; [i] $AlCl_3$, $LiAlH_4$, THF, Et_2O; [j] Raney Ni, EtOH.

The resolution of PD 135385 (**18**), one of the most interesting compounds from this series, is outlined in Scheme 2.[14] All attempts to resolve PD 135385 or any of its possible synthetic precursors (such as 4-phenylcyclohex-3-enylacetic acid) *via* diastereomeric salts failed to provide enantiomerically enriched materials. All attempts to resolve 4-phenyl-cyclohex-3-enecarboxylic acid or 4-phenylcyclohex-3-enylacetic acid failed to give diastereomeric salts that could be recrystallized to a constant rotation. It was postulated that increasing the asymmetry of the molecule would facilitate resolution of one of the possible synthetic intermediates. This was readily achieved by addition of a bulky thioketal group to the structure of 4-phenylcyclohex-3-enylacetic acid. Treatment of 3-phenylcyclohexen-2-one (**9**) with LDA (-78 °C) and ethyl bromoacetate gave ketoester **10**. Thioketal formation and ester hydrolysis produced the desired acid **11**. Recrystallization of the salt of **11** with either (**R**)-(-) or (**S**)-(+)-phenylglycinol provided about 25% yields (50% of theoretical) of (**R**) or (**S**)-**12**, respectively, with high enantiomeric purity.[15] The side chain of these compounds was elaborated by amide formation with 1-(2-pyridyl)piperazine, followed by reaction with $AlCl_3/LiAlH_4$; thioketal reduction with Raney nickel in ethanol provided (**S**)-(-)-PD 135385 (PD 137789) and (**R**)-(+)-PD 135385 (PD 137821), respectively.

Pharmacology

Compounds were tested for their ability to bind to rat striatal DA D_1 and D_2 receptors *in vitro*[16] and to inhibit rat brain DA synthesis *in vivo*. Under the test conditions, DA agonists block the increase in DA synthesis produced by γ-butyrolactone (GBL).[17] DA agonists also inhibit brain DA neuronal firing;[18] this inhibition was used to corroborate the DA agonist activity of selected compounds. Behaviorally, DA autoreceptor agonists have been shown to selectively inhibit spontaneous locomotor activity (LMA) in rodents placed in a novel environment.[19] This behavior, which is also produced by classical DA antagonists, results from the disruption of brain dopaminergic neurotransmission. Highly efficacious DA agonists, like apomorphine, which stimulate DA autoreceptors at low doses, also produce locomotor stimulation and stereotyped behavior at higher doses, as a result of postsynaptic DA receptor stimulation. Thus, rodent locomotor activity and stereotypy provide valuable behavioral measures of DA autoreceptor selectivity.[19]

Results and Discussion

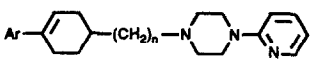
As shown in Table 1, compounds of general structure **Ia** and **Ic** (n=0 and 2, respectively) possess higher dopaminergic activity and oral efficacy than **Ib** (n=1). Thus, **14** and **19** both bind to D_2 receptors, inhibit mouse LMA, and quite effectively inhibit rat brain DA synthesis. On the other hand, the affinity of **16** and **17** for D_2 receptors is much weaker, and they produced none of the behavioral effects associated with dopaminergic activity. This periodical phenomenum has been described previously for a different group of DA agonists.[20] In general, compounds **Ia** and **Ic** bind to D_2 receptors with moderate affinity when displacing the DA antagonist [^{3}H]-spiperone. However, as shown in Table 2, these compounds displace [^{3}H]NPA with greater potency, an effect that is typical of DA agonists.

Compound **18** (PD 135385) was selected, because of its overall biochemical and behavioral profile, for more in-depth study. While this compound was not the most potent DA agonist from this series, its ability to inhibit rodent LMA selectively, with no stimulation of LMA even at the highest dose tested (100 mg/kg), identified it as a partial DA agonist with potentially selective DA autoreceptor agonist activity *in vivo*.

The dopaminergic activity of the enantiomers of **18** was evaluated separately (Table 2). The selectivity of both enantiomers for D_2 versus D_1 DA receptors was comparable (about tenfold, if we compare the values obtained with the D_1 and D_2 antagonists [^{3}H]-SCH 23390 and [^{3}H]-spiperone, respectively). Both enantiomers were equally active in the rat LMA inhibition test, with no locomotor stimulation observed in the dose-range tested (up to 100 mg/kg). Biochemically, both enantiomers possessed similar ability to inhibit GBL-induced rat brain DA synthesis. A slight difference in potency was observed in the DA neuronal firing test, with (+)-**18** (PD 137821) possessing greater activity than (-)-**18** (PD 137789) at the test dose of 2.5 mg/kg. Thus, both enantiomers of **18** appear to possess agonist activity at D_2 receptors. The lack of postsynaptic stimulation in rodents suggested that

both compounds might possess the appropriate level of partial D_2 agonist activity to be considered as selective DA autoreceptor agonists. However, lack of LMA stimulation may not necessarily reflect DA autoreceptor selectivity. The postsynaptic effects of high efficacy, selective D_2 agonists are generally difficult to detect in behavioral tests. Concurrent stimulation of D_1 and D_2 receptors is required, for instance, to produce a full profile of stereotypic behavior in rodents.[21] This type of test was used to differentiate between the enantiomers of **18**: (-)-**18** was tested for stereotypy in rats at a dose ten times the ED_{50} for inhibition of LMA, while (+)-**18** was tested at a dose 12.7 times the ED_{50} for inhibition of LMA. Both compounds were co-administered with a dose of the selective D_1 agonist SKF-38393 that does not by itself produce stereotypy. While (±)-**18** and (-)-**18** did not induce any stereotypy, (+)-**18** produced stereotypy in 60% of the rats tested (n=6).

Table 1. Structure-activity relationships for compound **I**.



Compound	n	Ar	[^{3}H]Spip[a] Binding,[b] IC_{50} (nM)	Inhibn. LMA:[c,d] ED_{50} (mg/kg) Mouse, ip	Rat, po	% Reversal of Rat Brain DA Synthesis[e] at 10 mg/kg, ip
13 (PD 135478)	0	Phenyl	600	1.0	>30	--
14 (PD 135222)	0	2-Pyridyl	1180	0.9	5.8	75 ± 4.3
15 (PD 135188)	0	2-Thienyl	352	9.3	>30	--
16 (PD 137012)	1	Phenyl	~10,000	~30	--	--
17 (PD 135146)	1	2-Thienyl	2430	>30	--	--
18 (PD 135385)	2	Phenyl	412	2.1	6.7	50 ± 0.9
19 (PD 135540)	2	2-Pyridyl	128	0.4[f]	~3.0[g]	89
20 (PD 135111)	2	2-Thienyl	436	8.4	13.0	46 ± 6.7

[a]Spip = Spiperone. [b]Screening data: IC_{50} values were obtained from 4 or 5 concentrations run in triplicate. [c]LMA = Locomotor activity. [d]ED_{50} values were generated from 4-6 doses, 5-12 animals per dose. [e]Shown is the percent reversal of the increase in DOPA levels in the striatum of GBL-treated rats (±SEM; n =4-5 animals). Endogenous levels of DA were not affected by the test compounds. [f]Produced stimulation at > 30 mg/kg. [g]Produced stimulation at > 10 mg/kg.

Table 2. Dopaminergic activity of the enantiomers of PD 135385.

Compound	DA Receptor Binding: IC_{50} (nM)[a] [^{3}H]Spip[b]	[^{3}H]NPA[c]	[^{3}H]SCH 23390[d]	Inhibn. LMA in Rat: ED_{50} (mg/kg, po)	Reversal of DA Synth. in Rat: ED_{50} (mg/kg, ip)	Inhibn. of DA Neuronal Firing in Rat at 2.5 mg/kg, ip	% Rats with Stereotypy[e] (dose, mg/kg, route)
(±)-**18** (PD 135385)	412 ± 22	88 ± 6	>6000	6.7	50 ± 1% at 10 mg/kg, ip	41 ± 13% (n=2)	0% (67, po)
(S)-(-)-**18** (PD 137789)	704 ± 208	82 ± 7	>6000	6.1	49 ± 2% at 10 mg/kg, ip	38 ± 13% (n=6)	0% (61, po)
(R)-(+)-**18** (PD 137821)	545 ± 108	64 ± 4	>6000	9.1	57 ± 8% at 10 mg/kg, ip	58 ± 6% (n=2)	60% (116, po)
Apomorphine	24 ± 4	1.7 ± 0.3	238	>30 (po), 0.03 (sc)	0.3	100%[f] (n=4)	100% (0.3, sc)[g]
(±)-**PD 128483**	2920 ± 331	202 ± 24	>10000	0.8	0.7	99 ± 1% (n=3)	0% (20, po)

[a]IC_{50} values were obtained from 4 or 5 concentrations, run in triplicate, by a non-linear regression analysis. [b]Spip = Spiperone; D_2 antagonist binding. [c]NPA = *N*-propylnorapomorphine; D_1/D_2 agonist binding. [d]D_1 antagonist binding. [e]Test compounds were coadministered with 10 mg/kg, sc, SKF 38393 (D_1 agonist). [f]100% inhibition of DA neuronal firing at 0.25 mg/kg, ip. [g]Apomorphine produced stereotypy without coadminstration of SKF 38393.

These results suggest that there is a difference in intrinsic activity between the enantiomers and that (-)-**18** is the less efficacious of the two. The partial D_2 agonist activity of (-)-**18** was confirmed by the experiment illustrated in Figure 1. *In vivo* treatment of rats with 1-carboethoxy-2-ethoxy-1,2-dihydroquinoline (EEDQ) has been shown to irreversibly alkylate and inactivate striatal DA autoreceptors.[22] Under alkylation conditions that leave at least 30% of DA autoreceptors intact, full DA agonists, such as N-propylnorapomorphine, can continue to elicit the same maximal effects (in this case, inhibition of brain DA synthesis) as in the absence of EEDQ, although higher doses of the agonist are required. However, the maximal response of a partial DA agonist such as (-)-**18** is clearly reduced versus the EEDQ-untreated controls. As seen in Figure 1, the maximal inhibition of striatal DA synthesis produced by (-)-**18** in EEDQ-treated rats (6 mg/kg) is only about 60% of that produced in EEDQ-untreated animals. In addition, higher doses of the compound are required to reach this maximal effect (ED_{50} values of 14 and 48 mg/kg, respectively). A similar situation has been described for the partial D_2 agonist EMD 23448.[22] These results confirm the partial DA agonist nature of (-)-**18**.

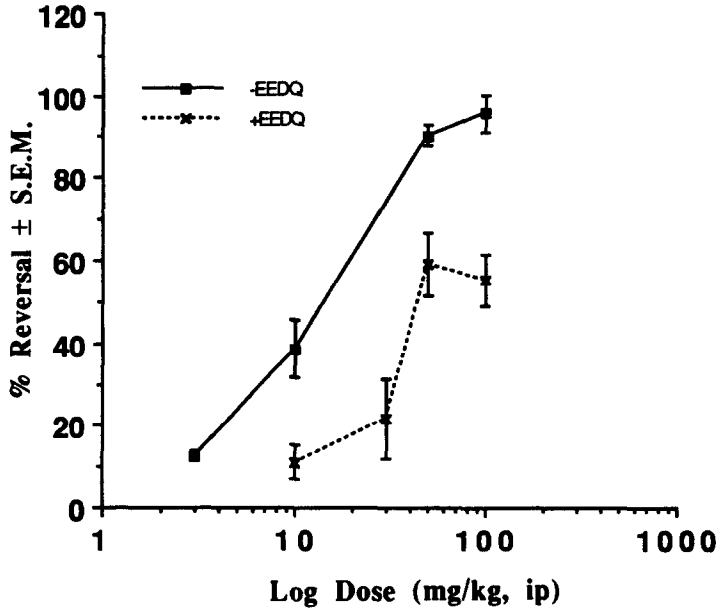


Figure 1. Reversal of GBL-induced striatal DOPA accumulation in control and EEDQ-treated animals. Groups of rats were treated with EEDQ (6 mg/kg sc) or vehicle. Forty-eight hours later all rats received a dose of the DOPA decarboxylase inhibitor NSD 1015 (100 mg/kg ip) 30 min prior to sacrifice. Some animals also received GBL (750 mg/kg ip; 30 min prior to sacrifice) alone or in combination with (-)-**18** (60 min prior to sacrifice). Each point is the mean of four rats.

In conclusion, these results confirm previous reports that selective *in vivo* DA autoreceptor activation can be achieved with partial DA D_2 agonists.[23] As shown here, it is essential to identify agents with the appropriate level of intrinsic activity. This is illustrated by the ability of (+)-**18**, a partial D_2 agonist, to produce stereotyped behavior in rats. Even though the difference in intrinsic activity between the two enantiomers of (±)-**18** is probably very small, (-)-**18**, the less efficacious of the two, appears to possess the expected activity profile of a selective DA autoreceptor agonist. Its good oral activity and CNS penetration further suggest that it may be an useful agent for the treatment of schizophrenia.

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