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Tetrahydrocarbazole-based serotonin reuptake inhibitor/dopamine D_2 partial agonists for the potential treatment of schizophrenia

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ABSTRACT

A 5-fluoro-tetrahydrocarbazole serotonin reuptake inhibitor (SRI) building block was combined with a variety of linkers and dopamine D_2 receptor ligands in an attempt to identify potent D_2 partial agonist/SRI molecules for treatment of schizophrenia. This approach has the potential to treat a broader range of symptoms compared to existing therapies. Selected compounds in this series demonstrate high affinity for both targets and D_2 partial agonism in cell-based and in vivo assays.

Schizophrenia is a complex psychiatric disorder that has been estimated to occur in up to 1% of the world's population.[1] There are three distinct symptom domains associated with the disease. Positive symptoms (e.g., delusions and hallucinations) are treated with atypical and typical antipsychotics such as olanzapine or haloperidol, respectively, by antagonizing excessive activation of dopamine D_2 receptors in the mesolimbic dopamine pathway. However, efficacy against negative (e.g., social withdrawal), cognitive (e.g., lack of ability to concentrate) and co-morbid mood symptoms with these agents is limited at best, and treatment of these aspects of the disease often requires additional pharmacotherapy.

In addition to a lack of efficacy against multiple symptom domains, typical antipsychotics such as haloperidol have adverse event profiles that limit their utility, including extrapyramidal effects (EPS) associated with excessive D_2 receptor antagonism in nigrostriatal regions of the brain.[2] Atypical antipsychotics such as clozapine and olanzapine exhibit a reduced EPS risk due in part to activity at other monoamine receptors in the CNS, such as 5-HT_{2A} antagonism, but have hematological and/or metabolic drawbacks.[2] Clinical evidence suggests that partial agonism at dopamine D_2 receptors confers antipsychotic efficacy with reduced EPS risk, and that combining dopaminergic activity with serotonin reuptake inhibition (SRI) can treat the negative symptoms associated with schizophrenia.[3] It has been reported that a dual acting D_2 antagonist/SRI shows activity in preclinical antipsychotic models.[4] Taking all of these factors into account, we hypothesized that combining dopamine D_2 *partial* agonism and SRI activity in a single molecule as a treatment for schizophrenia may be an attractive approach for the discovery of innovative antipsychotics.

In this Letter, we describe our initial efforts to achieve this goal and show that it is possible to identify molecules with dual D_2/SRI activity that show D_2 partial agonism in vitro, with antipsychotic activity and the potential for lower risk of EPS side effects in rodent models of dopaminergic function.

A known SRI pharmacophore, 5-fluoro-tetrahydrocarbazole **1**[5] was combined with D_2 fragments **2**[5,7] and related piperazines **3** and **4**[4] (Fig. 1). These D_2 ligands were selected because they furnished compounds with partial (**5**)[6] and full D_2 antagonist activity.[4] We envisioned the use of flexible and conformationally restricted linkers attached to the tetrahydrocarbazole fragment at C-2 or C-3 to evaluate the effect these linkers might have on D_2 functional activity in target molecules. In addition, the potential of the linker to influence SRI and D_2 binding affinity was of interest.

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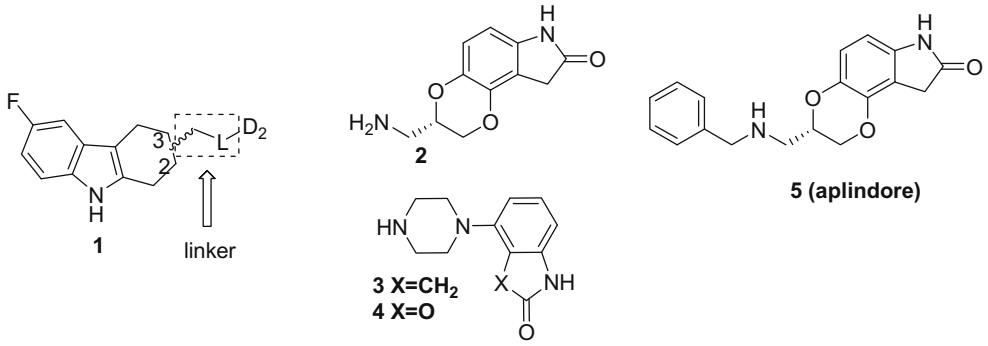
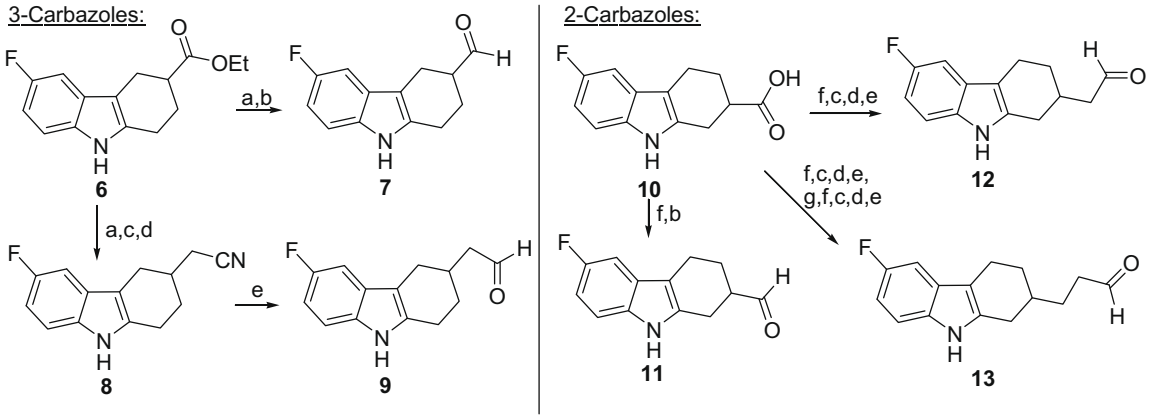


Figure 1. Design of partial D_2 agonist/SRI molecules.

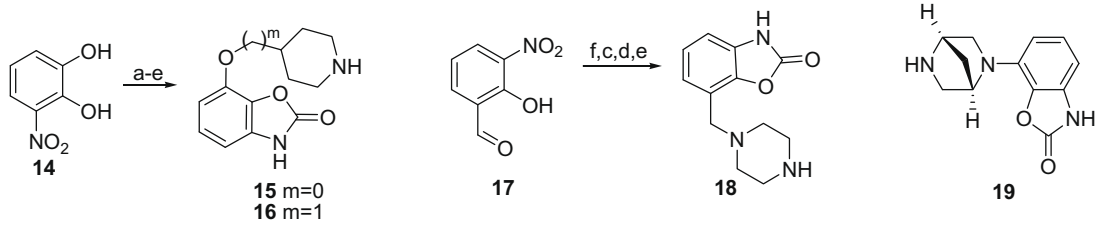
The synthesis of alkyl-linked tetrahydrocarbazole building blocks **7**, **9** and **11–13** is outlined in Scheme 1. Optically pure benzyl amine **5** (aplindore), lactam **3** and oxazolone **4** were prepared by published methods.[4,7] D_2 building blocks **15**, **16** and **18** were synthesized as shown in Scheme 2. SRI fragments were coupled to aplindore and piperazinyl derivatives **3** and **4** by reductive amination (Scheme 3). Target compounds derived from **5** (**22–27**) were separated into pure diastereomers by preparative HPLC prior to debenzylation.[8] Enantiomers **30** and **31** were obtained by chiral HPLC.[8] Diastereomers **39** and **40**, obtained following reductive amination between **7** and **19**, were separated by preparative HPLC.[8] All other target molecules were tested as racemic mixtures.[8]

Target compounds were screened for binding at the human serotonin reuptake site in a [^{3}H]citalopram displacement assay.[9] D_2 receptor binding was measured using [^{3}H]spiperone as the ligand.[10] Compounds with K_i values less than 50 nM at both receptors advanced to an in vitro D_2 functional assay that measures changes in intracellular cAMP levels.[11] Agonist activity was defined as a ratio (α-value) relative to the full agonist quinpirole (α-value 1).[11] Preferred compounds exhibited α-values between 0.2 and 0.5 in the in vitro D_2 functional assay. Molecules that met SRI and D_2 affinity and functional activity criteria were tested in animal models reflective of each target's activity. SRI activity was evaluated acutely using ip administration (3, 10, 30 mg/kg) in a mouse 5-HTP potentiation model.[12] Apomorphine-induced climbing (AIC) versus stereotypy in mice and rat conditioned avoidance responding (CAR) were used as models to predict atypicality and anti-psychotic activity.[13]

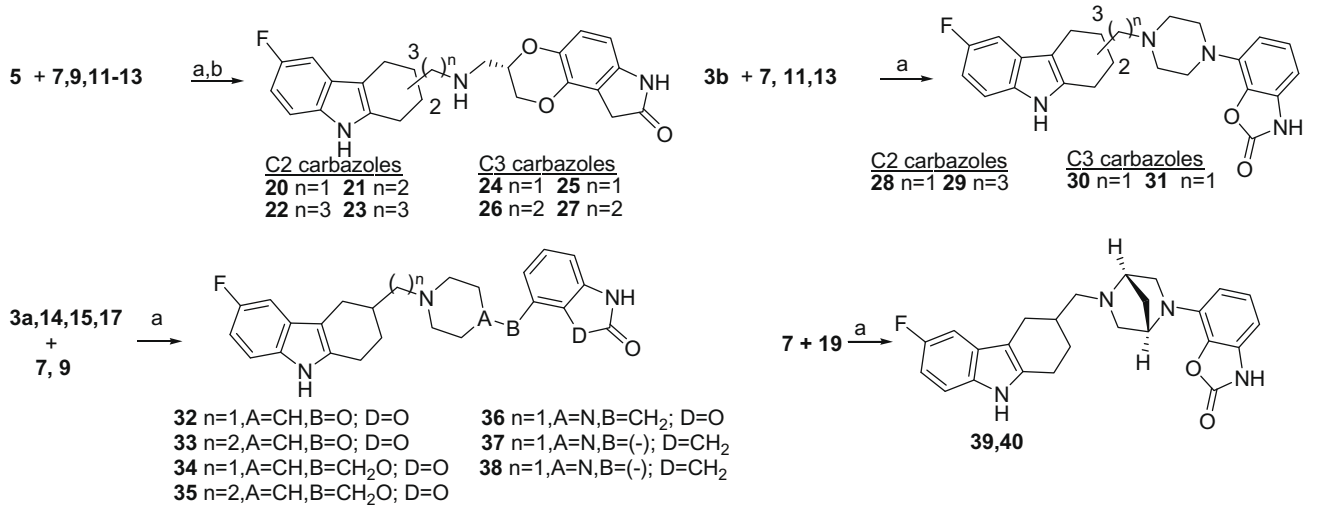
SRI and D_2 receptor K_i values are shown in Table 1. When comparing C2- versus C3-substituted carbazoles that contain aplindore-based D_2 moiety **2**, the C3 derivatives show higher SRI affinity than the corresponding C2 regioisomers. For SRI affinity, a single methylene unit is preferred for C3-analogs, while a propyl linker is preferred at C2. With the exception of **21**, the D_2 ligand has



Scheme 1. Reagents and conditions: (a) $LiAlH_4$/THF, rt; (b) Py-SO_3/TEA/DCM, rt; (c) MsCl/Et_3N/DCM, rt; (d) NaCN/DMF, 85 °C; (e) DIBAL/toluene, −78 °C; (f) BH_3/THF, 0 °C; (g) KOH/EtOH, reflux.



Scheme 2. Reagents and conditions: (a) 4-F-Benzyl bromide/$NaHCO_3$/DMF, rt; (b) *N*-Boc-*O*-Ts-piperidine/K_2CO_3/DMF, rt; (c) 40 psi H_2/10% Pd–C/EtOH; (d) CDI/THF reflux; (e) TFA/CH_2Cl_2; (f) Boc-piperazine/$Na(OAc)_3BH$/THF/1,2-dichloroethane, rt.



Scheme 3. Reagents and conditions: (a) $Na(OAc)_3BH$/THF/1,2-dichloroethane, rt; (b) 45 psi H_2/10% w/w $Pd(OH)_2$/MeOH–aq HCl.

Table 1
SRI and D_2 affinity

Compd	D_2	SRI K_i (nM)	D_2 K_i (nM)
2-Carbazoles			
20	**2**	25	100
21	**2**	5200	36
22	**2**	15	50
23	**2**	4	45
28	**3b**	62	220
29	**3b**	62	50
3-Carbazoles			
24	**2**	7	3
25	**2**	1	3
26	**2**	24	24
27	**2**	21	24
30	**3b**	32	60
31	**3b**	22	17
39	**30**	8	14
40	**30**	460	12
37	**3a**	1140	15
38	**3a**	4700	15
36	**17**	41	120
32	**14**	3	140
33	**14**	1200	310
34	**15**	0.4	28
35	**15**	10	720

Standards: Escitalopram SRI K_i 1 nM; haloperidol D_2 K_i 3 nM.
All K_i values represent averages of at least three independent experiments. α-Values represent average of at least two independent experiments.

Table 2
D_2 functional activity and preliminary receptor selectivity profile

Compd	D_2 functional activity (α-value)	5-HT_{2B} K_i (nM)	$5HT_{1A}$ K_i (nM) (function)	Rat α1 K_i (nM)
22	0.3	610	6	54
23	0.3	400	NT	22
24	0.3	130	2 (agonist)	0.5
25	0.4	6	2 (antag)	0.72
31	0.6	680	130 (partial agonist)	220
34	<0.1	110	NT	130
39	0.3	165	1800	65

All K_i values represent averages of at least three independent experiments. α-Values represent average of at least two independent experiments.

a modest, variable effect on SRI affinity in this group (**20**–**27**). SRI and D_2 affinity is similar in pairs of carbazole diastereomers (**22**/**23**, **24**/**25**) and enantiomers **30**/**31**. However, bicyclopiperazine diastereomers **39** and **40** display a substantial divergence in SRI affinity. Aplindore-based derivatives provide equal or higher SRI affinity compared to the oxazolone analogs **30** and **31**. Oxygen and carbon-linked piperidines and piperazine derivatives **32**–**38** showed variable D_2 affinity, and in most cases, lower SRI affinity compared to other compounds in Table 1. This data indicates that the linker can have a significant effect on receptor affinity, and collectively suggests a complex interplay between structure and activity in these multi-target molecules.

D_2 functional activity, 5-HT_{2B},[14] $5HT_{1A}$[14] and α1[15] affinity for compounds that met primary receptor affinity criteria are shown in Table 2. None of the compounds in this group displayed agonist activity in a $5HT_{2B}$ functional assay.[16] Aplindore derivatives **22**–**25**, and phenyloxazolone analog **31** showed an acceptable degree of D_2 partial agonism in vitro. It is interesting to compare the properties of **31** and **39** because they differ by a single methylene unit that fixes the flexibility of the piperazine ring. The bicyclic linker in **39** confers less D_2 agonist activity, and suggests that D_2 function can be influenced by the linker. Piperazine **31** shows decreased affinity for $5HT_{2B}$ and α1 compared to **39**, and increased $5HT_{1A}$ affinity. The compounds listed in Table 2, with the exception of **34** (a full D_2 antagonist), were tested in in vivo assays. In the 5-HTP model, only **31** (30 mg/kg ip) potentiated symptoms of serotonin syndrome. One possible explanation for the lack of activity of **22–25** and **39** in this assay is the less than 10-fold separation between SRI and α1 affinity. Antagonism of the α1 receptor is known to acutely suppress serotonin release in the CNS.[17] In the mouse AIC model, **39** demonstrated an atypical antipsychotic-like profile with separation between climbing and stereotypy effects (climbing ED_{50} 2.3 mg/kg ip, stereotypy ED_{50} of >5 mg/kg ip). In a mouse catalepsy model, **39** showed 48% catalepsy at 10 mg/kg (ip) at 90 min. Piperazine **31** demonstrated atypicality in the AIC model (climbing ED_{50} 0.5 mg/kg ip, stereotypy ED_{50} >1 mg/kg ip) and it was devoid of cataleptogenic potential at doses up to 10 mg/kg over a similar time frame. In the rat CAR model, **31** showed activity following ip and oral (10 mg/kg) administration by reducing avoidance response without an increase in escape response. Bicyclopiperazine **39** was not tested in the CAR model because of its less favorable receptor binding (α1) and in vivo activity profile (catalepsy, lack of 5-HT potentiation), compared to **31**.

In summary, we demonstrated that dual acting SRI and D_2 compounds with high affinity at both receptors can be synthesized. Selected compounds demonstrated D_2 partial agonism, a property that may contribute to effective treatment of the positive symptoms of schizophrenia with a reduced risk of EPS. Receptor binding and functional activity measured with these compounds indicates a complex relationship between structure and these properties. Future studies in this area will address receptor selectivity and pharmacokinetic/pharmacodynamic properties of partial D_2/SRI-active molecules in an attempt to provide proof of principle tools for this interesting concept in schizophrenia treatment.

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