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Dual D_2 and 5-HT_{1A} Receptors Binding Affinities of 1-Aryl-4-(diarylmethylene)piperazines and Piperidines

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Although atypical antipsychotics provide significantly greater efficacy against negative symptoms and cognitive deficits of schizophrenia, improvements in negative symptoms and cognition remain modest. The third generation antipsychotics which combine D_2 receptor blockade with 5-HT_{1A} receptor activation appear to provide therapeutic benefits against a broader range of symptoms and are essentially free of EPS liability. In an ongoing effort, we have identified new dihydroquinoline, tetrahydroquinoline and methoxyquinoline derivatives of 1-aryl-4-(diarylmethylene)piperazines and 4-aryl-1-(diarylmethylene)piperidines, which are structural analogs of adoprazine (SLV313). The described compounds have been screened for binding affinities of D_2 and 5-HT_{1A} receptors. The structure-activity relationship studies indicated that cyclopentenylpyridine, fluorophenylpyridine and cyclopentenylbenzyl groups significantly contribute to the high-binding affinities of these compounds to D_2 and 5-HT_{1A} receptors.

Keywords: 1-Aryl-4-(diarylmethylene)piperazine, 4-Aryl-1-(diarylmethylene)piperidine, Antipsychotics, D_2 and 5-HT_{1A} receptor.

INTRODUCTION

Schizophrenia is a severe psychiatric illness afflicting 1 % of the population worldwide. Diagnosis is based on diverse and variably expressed symptoms which include positive symptoms such as disorganized thought, delusions and auditory hallucinations, as well as negative symptoms for instance emotional flattening, poverty of speech and motivational deficits[1]. Various molecular mechanisms have been proposed to provide antipsychotic activity[2], but antagonism of the dopamine D_2 receptor subtype remains the cornerstone of antipsychotic activity[3,4]. The first-generation antipsychotics, or typical antipsychotics such as chlorpromazine (**1**) and haloperidol (**2**) are dopamine antagonist and exhibits robust control of positive symptoms of schizophrenia, such as hallucinations, agitation and delusions but fail to control the negative symptoms for instance blunted affect, emotional withdrawal, cognitive deficits, apathy and in addition motor retardation remain uncontrolled with these therapeutics for most schizophrenic patients[5]. Moreover, selective D_2 receptor antagonists block nigrostriatal dopaminergic activity, leading to extrapyramidal symptoms (EPS) including dystonia and dyskinesia and also to the blockade of pituitary-located D_2 receptors that control prolactin release, leading to hyperprolactinemia[6]. The 'second-generation' or atypical antipsychotics, such as clozapine, combine D_2 receptor antagonism with activity at other receptors, on the premise that a suitable balance of pharmacological activity should broaden the spectrum of therapeutic efficacy and reduce extrapyramidal symptoms. With respect to classical neuroleptics, clozapine shows significantly greater efficacy, including an improved effect on negative symptoms and causes a marked increase in dopamine output in the prefrontal cortex[7]. Although 5-HT_{2A} antagonism is a prominent feature of atypical antipsychotics[8], improvements in negative symptoms and cognition remain modest.

To achieve improved overall therapeutic benefit in the management of schizophrenia, combining D_2 receptor blockade with 5-HT_{1A} receptor activation rather than antagonism has been a subject of recent research attention[9,10]. Indeed, numerous mechanistic considerations[11-13] and preclinical evidence[14-16] support the potential of such a combination. Consequently adoprazine (**3**) (SLV-313) and bifeprunox (**4**) (Fig. 1), bearing potent D_2 receptor antagonist and 5-HT_{1A} receptor agonist properties, were developed[17].

However, the failure of compounds **3** and **4** to oppose phencyclidine-induced social interaction deficits suggested that an appropriate 'balance' of activity at these sites is necessary for activity in this model[18]. Thus, the need to discover compounds having varying ratios of D_2 and 5-HT_{1A} activities continued[19].

In search to discover new antipsychotics, we have recently synthesized a series of new quinoline, dihydroquinoline and methoxyquinoline derivatives of 1-aryl-4-(diarylmethylene) piperazines and 4-aryl-1-(diarylmethylene)piperidines (**5-26**), which are structural analogs of SLV313 (Fig. 2)[20,21]. Herein we wish to disclose dual D_2 and 5-HT_{1A} receptor binding affinities of these compounds and their structure-activity relationship.

EXPERIMENTAL

Chemical synthesis: All the target compounds **5-26** were synthesized by adopting known procedures[20,21].

Radioligand binding assays

Rat-cloned D_{2L} dopaminergic receptors: Human cloned dopamine D_{2L} receptors stably expressed in C6 rat glioma cells were radiolabelled with [^{3}H] spiroperidol according to Scarselli *et al*[22]. with minor modifications. The incubation buffer (120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 50 mM *tris*-HCl, pH 7.4) contained 100 µg of dopamine D_{2L} receptor membranes, 0.30-0.50 nM [^{3}H] spiroperidol (K_d = 0.093 nM) and 6 to 9 concentrations of drug solution in a final volume of 500 µL. The samples were incubated for 120 min at 25 °C, then the incubation was stopped by rapid filtration through

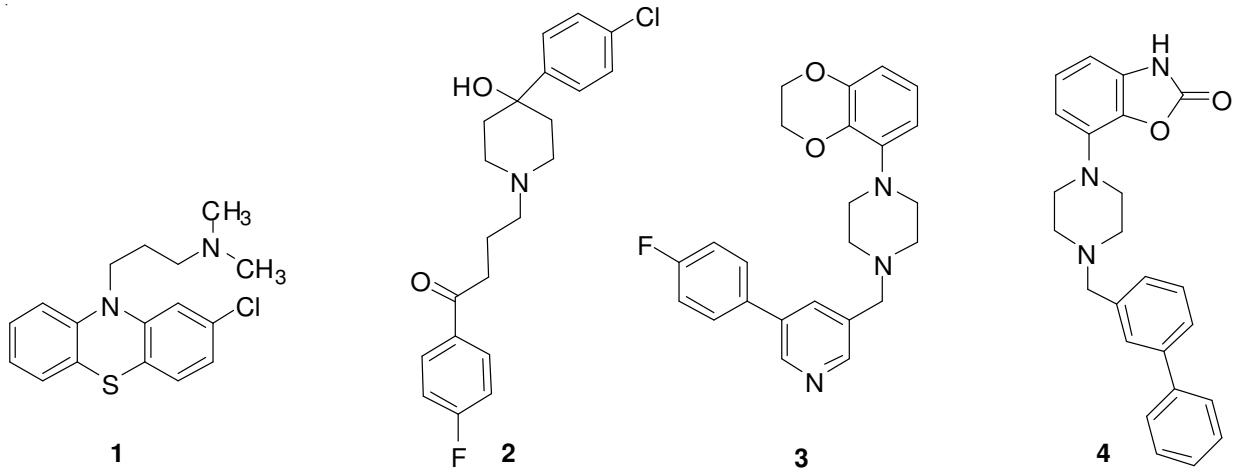
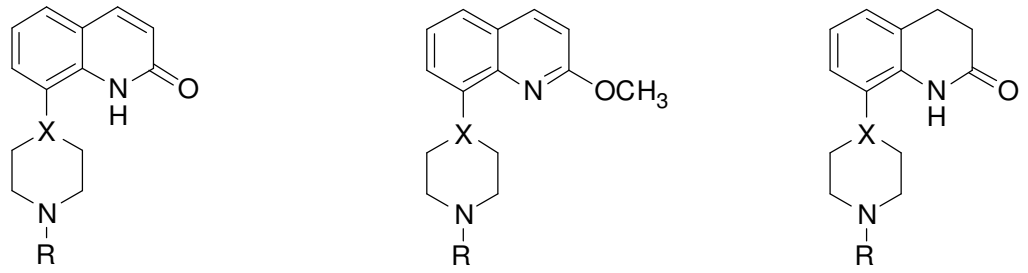


Fig 1. (1) Chlorpromazine, (2) haloperidol, (3) adoprazine and (4) bifeprunox



Piperazinyl analogues

(**5**) X = N; R = **a**	(**8**) X = N; R = **d**	(**12**) X = N; R = **d**
(**6**) X = N; R = **b**	(**9**) X = N; R = **c**	(**13**) X = N; R = **c**
(**7**) X = N; R = **c**	(**10**) X = N; R = **a**	(**14**) X = N; R = **b**
	(**11**) X = N; R = **b**	(**15**) X = N; R = **a**

Piperidinyl analogues

(**16**) X = C; R = **a**	(**23**) X = C; R = **c**	(**20**) X = C; R = **a**
(**17**) X = C; R = **b**	(**24**) X = C; R = **d**	(**21**) X = C; R = **d**
(**18**) X = C; R = **c**	(**25**) X = N; R = **a**	(**22**) X = C; R = **c**
(**19**) X = C; R = **d**	(**26**) X = C; R = **b**	

R =

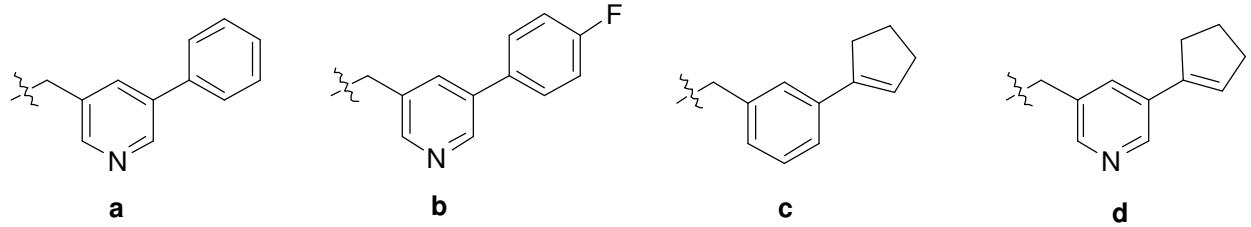


Fig. 2. Chemical structures of compounds **5-26**

Whatman GF/C glass fiber filters (presoaked in 0.5 % polyethylenimine for 60 min). The filters were washed 3 5 1 mL of ice-cold 50 mM *tris*, 0.9 % NaCl, pH 7.4. Non specific binding was determined in the presence of 10 µM haloperidol. The radioactivity bound to the filters was measured by liquid scintillation using LS6500 Multi-Purpose scintillation Counter, Beckman.

Human-cloned 5-HT$_{1A}$ receptors: Human cloned 5-HT$_{1A}$ serotonin receptors stably expressed in HEK293-EBNA cells (Perkin-Elmer, Waltham, MA, USA) were radiolabelled with 1 nM [^{3}H]-8-OH-DPAT[23]. Samples containing 32 µg of membrane protein, different concentrations of each compound ranging from 0.1 to 10 µM were incubated in a final volume of 500 µL of 50 mM *tris*-HCl pH 7.4, 5 mM $MgSO_4$ for 120 min at 37 °C. After this incubation time, samples were filtered through Whatman GF/C glass microfiber filters pre-soaked in polyethylenimine 0.5 % for at least 0.5 h prior to use. The filters were washed twice with 1 mL of ice-cold buffer (50 mM *tris*-HCl, pH 7.4). Nonspecific binding was determined in the presence of 10 µM 5-HT. The radioactivity bound to the filters was measured by liquid scintillation using LS6500 Multi-Purpose scintillation Counter, Beckman.

Determination of K$_i$ values: K$_i$ values of the compounds were calculated by the Cheng-Prusoff equation[24] and are expressed as means ± SEM of separate experiments each performed in duplicate.

RESULTS AND DISCUSSION

The 1-aryl-4-(diarylmethylene)piperazines and 4-aryl-1-(diarylmethylene)piperidines that each constitute quinoline, methoxy and dihydroquinoline derivatives showed dual binding affinities for D_2 and 5-HT$_{1A}$ receptors as shown in Table-1. These compounds in general possessed higher 5-HT$_{1A}$ receptor affinities than that of D_2 receptor.

TABLE-1
STRUCTURES AND AFFINITIES (K$_i$, nM) OF COMPOUNDS **5-26** ON D_2 AND 5-HT$_{1A}$ RECEPTORS. BINDING AFFINITY VALUES ARE EXPRESSED AS MEANS ± SEM OF SEPARATE EXPERIMENTS, EACH PERFORMED IN DUPLICATE

Piperazinyl ligand	K$_i$ ± SEM		Piperazinyl ligand	K$_i$ ± SEM	
	D_2	5-HT$_{1A}$		D_2	5-HT$_{1A}$
Haloperidol	2.92 ± 0.7	–	**15**	28.4 ± 1.5	4.30 ± 0.3
5-HT		10.0 ± 1.2	**16**	500.0 ± 32.0	4.43 ± 0.6
5	144.0 ± 9.0	4.04 ± 0.7	**17**	67.6 ± 4.0	3.58 ± 0.5
6	7.53 ± 0.8	4.90 ± 0.3	**18**	1.64 ± 0.5	3.93 ± 0.5
7	71.4 ± 5.0	2.97 ± 0.8	**19**	17.4 ± 1.1	11.3 ± 1.3
8	34.6 ± 5.0	2.77 ± 0.9	**20**	14.1 ± 1.2	4.00 ± 0.8
9	12.2 ± 2.0	0.97 ± 0.3	**21**	8.56 ± 0.8	4.15 ± 0.3
10	16.9 ± 2.0	3.10 ± 0.4	**22**	102.0 ± 6.0	3.75 ± 0.8
11	33.6 ± 3.0	2.86 ± 0.7	**23**	524.0 ± 29.1	2.13 ± 0.7
12	2.16 ± 0.4	28.3 ± 1.5	**24**	500.0 ± 32.0	4.68 ± 1.0
13	36.7 ± 2.5	4.63 ± 0.6	**25**	19.0 ± 1.8	3.63 ± 0.2
14	42.0 ± 2.0	52.5 ± 4.0	**26**	5.68 ± 0.6	12.83 ± 1.1

Structure-activity relationship (SAR) of D_2 receptor binding of 1-aryl-4-(diarylmethylene)piperazines: The SAR studies on D_2 receptor binding within the series revealed interesting clues to refinement to relatively potent ligands. The most potent compounds in this series were compounds **12**, **6** and **9** compared to others with moderate affinity. Compound **5** exhibited weak affinity; however, addition of a fluoro group improved its D_2 receptor affinity to 19-fold yielding compound **6**. It was observed that addition of a fluoro group to quinoline derivatives contributes significantly to enhancing the binding affinity for the receptor. However, in case of methoxyquinoline derivatives (**10**) (K$_i$ = 16.9 nM) lost 2-fold affinity when derivatized with a fluoro group at the same position yielding compound **7** (K$_i$ = 33.6 nM); likewise in case of tetrahydroquinoline derivatives, fluorinated phenylpyridine analogue (**14**) lost 1.5-fold affinity compared to its non-fluorinated counterpart compound **15**.

As mentioned earlier compound **12** (tetrahydroquinoline derivative) was the most potent ligand for D_2 receptors in the series primarily due to the presence of a combination of tetrahydroquinoline and cyclopentenylpyridine substitution (K$_i$ = 2.16 nM). This is supported by the observation that the pyridine nitrogen potentially plays a major role in the high affinity of compound **12**, which when substituted with cyclopentenylpyridine moiety, yielding compound **13**, exhibited much lower affinity (K$_i$ = 36.7 nM). Comparison of hydroquinoline derivative compound **5** with its counterparts compound **10** (methoxyquinoline) and compound **15** (tetrahydroquinoline) showed a marked difference in the affinity profile. The affinities of compounds **10** and **15** were improved to more than 8 and 5-fold, respectively when compared with compound **5** indicating an important contribution of methoxyquinoline moiety along with the phenylpyridine substitution. Similar comparison strategy was applied to compound **6** but with a fluorophenylpyridine moiety which showed contrasting results. Adding a fluorophenylpyridine substitution to methoxy and tetrahydroquinoline derivatives (**11** and **14**) did not improve the affinity rather it decreased it many fold. Moreover (a methoxyquinoline derivative) (**9**) with a cyclopentenylbenzyl substitution carried highest binding affinity for D_2 receptors (K$_i$ = 12.2 nM) among its counterparts compounds **7** and **13**. It is interesting to note that in the methoxyquinoline series, the presence of pyridine nitrogen in compound **8** significantly decreased its affinity (K$_i$ = 34.6 nM) when compared to compound **9** of the same series that contained no such nitrogen (K$_i$ = 12.2 nM). However, the same pyridine nitrogen in compound **12** is a major enhancer of D_2 binding affinity in the tetrahydroquinoline series (K$_i$ = 2.16 nM) when compared to compound 13 that contains no such nitrogen (K$_i$ = 36.7 nM). The structure-activity relationship studies revealed that the combination of pharmacophores required to maximize the D_2 binding affinity among the piperazine series include: tetrahydroquinoline + cyclopentenylpyridine; dihydroquinolone + fluorophenylpyridine and methoxyquinoline + cyclopentenylbenzyl derivatizations. Identification of these pharmacophores provides important clues to further tailoring and refinement of the compounds in order to develop potent D_2 receptor binding drugs.

Structure-activity relationship of 5-HTS$_{1A}$ receptor binding of 1-aryl-4-(diarylmethylene)piperazines: The compounds exhibited higher affinity to 5-HT$_{1A}$ receptor than that of D_2. The K$_i$ values of most of the compounds ranged from 0.97 to 52.5 nM. Compound **9** was the most potent of the series (K$_i$ = 0.97 nM) indicating that methyoxyquinoline with a cyclopentenylbenzyl substitution is possibly a right

combination for higher affinity. This is confirmed by comparing it with its counterparts compounds **7** and **13** that possessed the same substitution but with dihydroquinoline and tetrahydroquinoline groups respectively, with lower affinity to the receptor. Compounds **5**, **10** and **15** all with cyclopentenylbenzyl derivative possessed stronger 5-HT_{1A} affinities than that of D_2. As discussed earlier, addition of a fluoro group to compound **6** significantly enhanced its D_2 activity. However, presence of the same group did not affect the 5-HT_{1A} affinity of compound **6** (K_i = 4.9 nM) when compared with compound **5** (K_i = 4.04 nM). In fact, the fluoro group caused a decrease in the affinity of **14** (K_i = 52.5 nM) leaving compound **11** to be the most active among its counterparts (K_i = 2.86 nM). Furthermore, the presence of pyridine nitrogen did not play a significant role in improving the binding; it reduced the 5-HT_{1A} affinities of all piperazine derivatives in contrast to that of D_2. In general, the dihydroquinoline and methoxyquinoline derivatives exhibited more potent activity for 5-HT_{1A} than the tetrahydroquinoline ones. Important pharmacophores identified from the piperazine series that are essential for 5-HT_{1A} binding affinity include methoxyquinoline and cyclopentenylbenzyl substitution (Table-1).

Structure-activity relationship of D_2 receptor binding of 4-aryl-1-(diarylmethylene)piperidines: Compounds in this series that possessed relatively higher binding affinity were compounds **18**, **26** and **21**. The phenylpyridine moiety in the dihydroquinoline series is not an ideal pharmacophore for D_2 affinity as in compound **16**, which has weakest affinity in the series. However, the activity dramatically increased by more than 35 and 26-fold when the same substitution was made to compounds **20** and **25** of the tetrahydroquinoline series, respectively. Compound **26**, a fluorophenylpyridine-substituted methyoxyquinoline, exhibited much higher affinity (K_i = 5.68 nM) than its dihydroquinoline counterpart **17** (K_i = 67.6 nM).

Attempts were made to further refine the activity of compound **18** by performing a number of structural modifications within the dihydroquinoline series as well as among the other series. These modifications include addition of a pyridine ring that adds only a nitrogen atom to the compound (as in **19**), which caused a 10-fold decrease in the binding affinity (K_i = 17.4 nM). However, when the same substitution was made to tetrahydroquinoline derivative, which yielded compound **21** with higher affinity (K_i = 8.56 nM) than that of compound **19**. However, methoxyquinoline derivative did not show significant activity when substituted with cyclopentenylpyridine moiety (as in **24**). The cyclopentenylbenzyl substitution was also performed in tetrahydro and methoxyquinoline series that yielded compound **22** and **23** with much lower affinity than that of compound **18**; in fact it decreased the activity several fold.

The structure-activity relationship studies within the piperidine class of compounds indicated important combinations of pharmacophores that are essential for higher binding affinity to D_2 receptor. These include quinoline + cyclopentenylbenzyl; tetrahydroquinoline + cyclopentenylpyridine and methoxyquinoline + fluorophenylpyridine derivatizations. General comparison between the potent compounds from the piperazine and piperidine derivatives clearly indicated that cyclopentenylpyridine / benzyl and fluorophenylpyridine groups play a major role in the binding affinity to D_2 receptor.

Structure-activity relationship of 5-HT_{1A} receptor binding of 4-aryl-1-(diarylmethylene)piperidines: The piperidine derivatives showed higher binding affinity toward 5-HT_{1A} receptor than D_2 receptor. The K_i values of most of the compounds ranged from 2-4 nM. The narrow K_i range most likely indicates that the 5-HT_{1A} receptor recognizes the general structures of dihydroquinoline, tetrahydroquinoline and methoxyquinoline derivatives since the activity differences between the derivatives are subtle. This is unlike D_2 receptor binding behaviour of the same compounds where slight changes in the substitutions caused marked differences in the affinity profile. Structure-activity relationship studies on the piperazine and piperidine derivatives for 5-HT_{1A} receptor binding indicated that generally all derivative types play some role in the higher binding affinity especially the cyclopentenylbenzyl substitution.

Conclusion

In a summary, a series of dihydroquinoline, tetrahydroquinoline and methoxyquinoline derivatives of 1-aryl-4-(diarylmethylene)piperazines and 4-aryl-1-(diarylmethylene) piperidines have been evaluated for binding affinities for D_2 and 5-HT_{1A} receptors. The structure-activity relationship of the described compounds revealed that dihydroquinoline and methoxyquinoline with cyclopentenylbenzene moiety is the right combination for D_2 and 5-HT_{1A} receptors optimal affinities, respectively. In general these compounds possessed higher 5-HT_{1A} receptor affinities than that of D_2 receptor and compounds with cyclopentenylbenzene and cyclopentenylpyridine moieties exhibited higher affinities for both D_2 and 5-HT_{1A} receptors.

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