Binding of Typical and Atypical Antipsychotic Agents to 5-Hydroxytryptamine-6 and 5-Hydroxytryptamine-7 Receptors[1]

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ABSTRACT

The authors examined the affinities of 36 typical and atypical antipsychotic agents for the cloned rat 5-hydroxytryptamine-6 (5-HT_6) and rat 5-hydroxytryptamine-7 (5-HT_7) receptors in transiently expressed COS-7 cells (5-HT_7) or stably transfected HEK-293 cells (5-HT_6 receptors). Clozapine and several related atypical antipsychotic agents (rilapine, olanzepine, tiospirone, fluperlapine, clorotepine and zotepine) had high affinities for the newly discovered 5-HT_6 receptor (K_is < 20 nM). The 5-HT_7 receptor bound clozapine, rilapine, fluperlapine, clorotepine, zotepine and risperidone but not tiospirone and olanzepine, with affinities less than 15 nM. In addition, several typical antipsychotic agents (chlorprothixene, chlorpromazine, clothiapine and fluphenazine) had high affinities for both the 5-HT_6 and 5-HT_7 receptors. Pimozide, a diphenylbutylpiperidine, had the highest affinity of all the typical antipsychotic agents tested for the 5-HT_7 receptor (K_i = 0.5 nM). Three putative atypical antipsychotic agents melperone, amperozide and MDL 100907 did not bind with high affinities to either the 5-HT_6 or 5-HT_7 receptors (K_is > 50 nM). Several dopamine-selective antipsychotic agents (raclopride, rimcazole and penfluridol) had essentially no affinity for either the 5-HT_6 or 5-HT_7 receptors (K_i values > 5000 nM). Although 5-HT_6 or 5-HT_7 receptor affinity alone does not predict whether or not a drug will have atypical antipsychotic activity, the relatively high affinity of the 5-HT_6 receptor for several clozapine-related compounds, in combination with the enrichment of 5-HT_6 messenger RNA in the striatum, suggests that the ability of at least some atypical antipsychotic drugs to interact with 5-HT_6 receptors may contribute to their lack of extrapyramidal side effects.

Clozapine, the prototypical atypical antipsychotic drug, has greater efficacy in treatment-resistant schizophrenia than do typical antipsychotic drugs (Kane *et al.*, 1988; Meltzer *et al.*, 1989a). In addition, unlike any other known antipsychotic agent, clozapine is effective in masking symptoms of tardive dyskinesia and tardive dystonia without appreciably causing other types of extrapyramidal symptoms (Meltzer and Luchins, 1984; Friedman and Lannon, 1989; Meltzer et al., 1989a; Lieberman *et al.*, 1991). The mechanism of action of clozapine remains unknown, although previous reports indicated that clozapine has functionally important interactions with serotonergic (Fink *et al.*, 1984; Meltzer *et al.* 1989b), dopaminergic (Altar *et al.*, 1988), muscarinic (Miller and Hilley, 1974) and *alpha*-1 adrenergic (Cohen and Lipinski, 1986) receptors. More recent studies examined the binding of clozapine and related compounds to the newly cloned D4 receptor (Van Tol *et al.*, 1991), the 5-HT_{1C} receptor (Canton *et al.*, 1990; Roth *et al.*, 1992) and all five subtypes of muscarinic receptors (Bolden *et al.*, 1992).

Since our previous study of the 5-HT_{1C} binding profile of clozapine and related atypical antipsychotic agents (Roth *et al.*, 1992), several additional 5-HT receptors have been cloned, including the 5-HT_{2F} (Kursar *et al.*, 1992), the 5-HT_{5A} and 5-HT_{5B} (Matthes *et al.*, 1993), 5-$HT_{1E\alpha}$ and 5-$HT_{1E\beta}$ (Amlaiky *et al.*, 1992; McAllister *et al.*, 1992; Zgombick *et al.*, 1992; Lovenberg *et al.*, 1993), 5-HT_6 (Monsma *et al.*, 1993) and 5-HT_7 (Shen *et al.*, 1993) receptors. Only the 5-HT_6 (Monsma *et al.*, 1993) and 5-HT_7 (Shen *et al.*, 1993) receptors were reported to bind clozapine with high affinities; scant published information is available in regard to the pharmacology of most of these newly cloned 5-HT receptor subtypes.

The 5-HT_6 and 5-HT_7 receptors pharmacologically resemble the 5-HT_2 receptor (Monsma *et al.*, 1993; Shen *et al.*, 1993), which has been postulated to be a major site of action of clozapine and related compounds (Meltzer *et al.*, 1989b). In particular, Monsma *et al.* (1993) and Shen *et al.* (1993) noted

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that both the 5-HT_6 and 5-HT_7 receptors appeared to have high affinities for many psychotherapeutic drugs of diverse classes (*e.g.*, antidepressants and antipsychotics). In addition, the 5-HT_6 receptor messenger RNA is highly enriched in striatum (Monsma *et al.*, 1993), a potentially important site of action of antipsychotic drugs with regard to both extrapyramidal symptoms and perhaps cognitive functioning.

For these reasons, we examined the binding affinities of 36 typical and atypical antipsychotic drugs for the rat 5-HT_6 and 5-HT_7 receptors by using both transient and stably expressing cell lines. We discovered that several atypical antipsychotic agents with high affinities for the 5-HT_2 receptor also possessed high affinities for both the 5-HT_6 and 5-HT_7 receptors. It is conceivable that certain unique properties of clozapine and related compounds could be mediated by functionally important interactions with either 5-HT_6 or 5-HT_7 receptors.

Materials and Methods

Materials. The sources of drugs used in this study were previously specified (Meltzer *et al.*, 1989b; Roth *et al.*, 1992) with the exception of the following compounds: olanzepine (Eli Lilly, Indianapolis, IN), MDL 100907 (Marion Merrell Dow, Cincinnati, OH), clorotepine (−) and clorotepine (+) (gifts of P. Seeman, Toronto, Canada), N-desmethyl and N-oxideclozapine metabolites (Sandoz, Basel, Switzerland). [^{3}H]-LSD (66 Ci/mmol) was from New England Nuclear (Boston, MA). Cell culture supplies were from GIBCO/BRL (Gaithersburgh, MD), common laboratory chemical were from Sigma (St. Louis, MO) and molecular biology reagents were obtained from Stratagene (Torry Pines, CA) or New England BioLabs (Boston, MA).

Cell culture. COS-7 cells were transfected with p5HT7 or pSVK3–5HT1C by using the DEAE-dextran technique of Cullen (Cullen, 1987) exactly as previously described (Choudhary *et al.*, 1992, 1993). The HEK-293 cells that express the 5-HT_6 receptor were previously characterized (Monsma *et al.*, 1993). The GF-6 cells, which stably express the 5-HT_2 complementary DNA, were from D. Julius and were previously characterized (Choudhary *et al.*, 1992, 1993). All cells were grown in Dulbecco's modified Eagle's medium, which contained high glucose (4500 mg/l), supplemented with glutamine, pyruvate and 10% fetal calf serum (regular medium). For binding experiments, the cells were switched to regular medium that contained 10% dialyzed medium at least 48 hr before harvesting.

Radioligand binding. The cells were washed with phosphate-buffered saline (148 mM NaCl, 2.7 mM KCl, 8 mM NaH_2PO_4, pH = 7.40, twice for 10 min each) and then harvested with a cell scraper into 20 ml of phosphate-buffered saline per plate. After concentration by centrifugation (1000 × *g* for 10 min), the cells were lysed in binding buffer (50 mM Tris Cl, pH = 7.40) and membranes were collected by centrifugation (20,000 × *g* for 20 min at 4°C). After resuspension and recentrifugation in binding buffer, the membranes were frozen as tight pellets at −80°C.

The 5-HT_2 and 5-HT_{1C} binding assays were performed exactly as previously described (Roth *et al.*, 1992; Choudhary *et al.*, 1992). For 5-HT_6 and 5-HT_7 receptors, [^{3}H]-LSD binding assays were performed in a total volume of 0.2 or 0.5 ml, respectively, at 25°C for 90 min in the dark, as previously detailed (Roth *et al.*, 1991, 1992). After incubation, the membranes were collected onto polyethyleneimine-pretreated glass fiber filters (GF/C, Whatman), washed with 5 ml of ice-cold binding buffer three times and then quantified by liquid scintillation spectrophotometry. Quenching was corrected for by the sample channels ratio technique. Nonspecific binding was determined with 10 μM clozapine and represented at least 95% of total binding. Competition and saturation binding data were analyzed by using the LIGAND program (Munson and Rodbard, 1980). The data reported here represent the mean of at least two separate competition binding studies in which five to seven concentrations of unlabeled ligand were used with duplicate determinations for each point. The protein concentration was determined with the Bio-Rad kit (Richmond, CA) by using bovine serum albumin as the standard.

Data analysis. The pK_i values of typical and putative atypical antipsychotic drugs were compared by *t* tests and correlated by using Spearman *rho* coefficient. The pK_i values were used as described in Meltzer *et al.* (1989b) to determine whether the 5-HT_6 and 5-HT_7 pK_i values contributed to the discrimination of typical and atypical antipsychotic drugs by using stepwise discriminative function analysis (Meltzer *et al.*, 1989b).

Results

Expression of rat 5-HT_6 and 5-HT_7 receptors. The HEK-293 cells that stably expressed the rat 5-HT_6 receptor were prepared as previously detailed (Monsma *et al.*, 1993). A

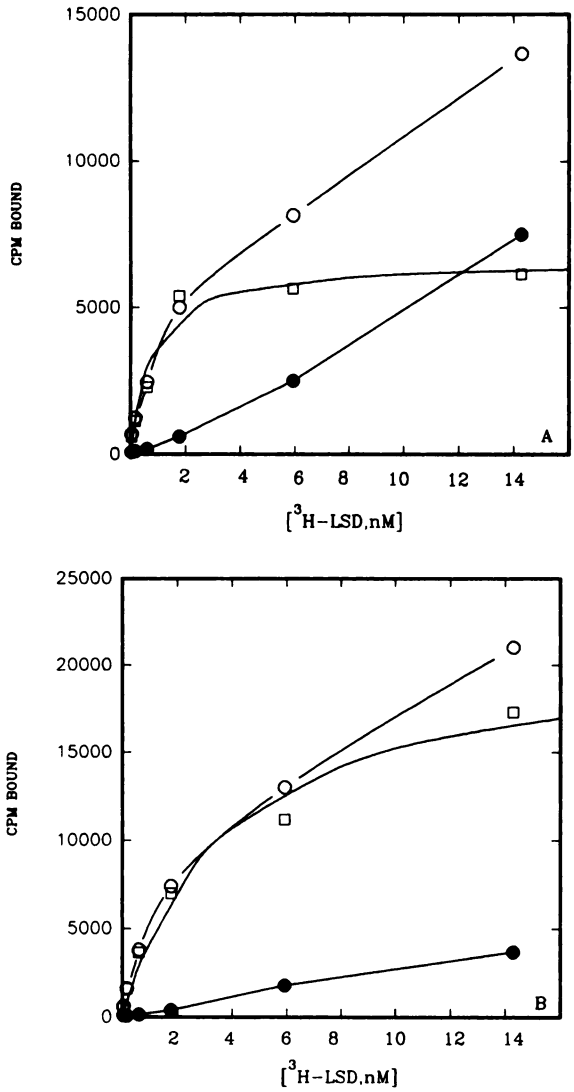


Fig. 1. Saturation binding plots of [^{3}H]-LSD to 5-HT_6 (A) and 5-HT_7 (B) receptors. Shown are typical saturation binding isotherms for [^{3}H]-LSD binding to 5-HT_6 (A) and 5-HT_7 (B) receptors. Shown are total (○), nonspecific (●) and specific (□) counts per minute bound at increasing [^{3}H]-LSD concentrations (in nanomolar quantities). The data were fit by using the LIGAND program; the lines were computer generated. Clozapine (10 μM) was used to define nonspecific binding. For the 5-HT_6 receptor, the following values were obtained (means ± S.E.M.): K_d = 1.3 ± 0.6 nm; B_{max} = 1.34 ± 0.3 pmol/mg. For the 5-HT_7 receptor, the following values were obtained (means ± S.E.M.): K_d = 7 ± 2.5 nM; B_{max} = 24.7 ± 4.3 pmol/mg.

line that expressed high levels of 5-HT_6 receptors was chosen for further study. As shown in figure 1A, [^{3}H]-LSD bound with high affinity and capacity to 5-HT_6 receptors stably expressed in HEK-293 cells. Untransfected HEK-293 cells do not express specific [^{3}H]-LSD binding (not shown). Figure 2 shows the time course of binding of [^{3}H]-LSD to the 5-HT_6 and 5-HT_7 receptors.

For 5-HT_7 receptors, transiently transfected COS-7 cells were used because they expressed higher receptor levels than did stably transfected HEK-293 cells. As shown in figure 1B, [^{3}H]-LSD bound to 5-HT_7 receptors transiently expressed in COS-7 cells. As a control, untransfected COS-7 cells do not express specific [^{3}H]-LSD binding (Roth *et al.*, 1992).

Binding of typical antipsychotic drugs to 5-HT_6 and 5-HT_7 receptors. Figure 3 shows that several typical antipsychotic drugs bound to 5-HT_6 receptors with high affinity.

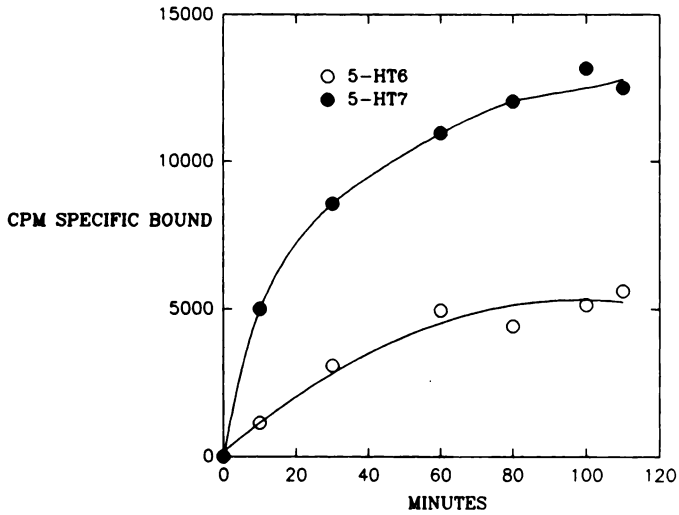


Fig. 2. Time course of [^{3}H]-LSD binding to 5-HT_6 and 5-HT_7 receptors. Shown is a typical time course experiment in which 5-HT_6 (○) or 5-HT_7 (●) receptors were incubated with 5 nM [^{3}H]-LSD at 25°C for various periods. Nonspecific binding was determined with 10 μM clozapine.

Tables 1 to 3 summarize our findings with 36 typical and atypical antipsychotic agents. For comparison, pK_i values for the 5-HT_2, 5-HT_{1C} and D2 receptors are also shown (tables 2 and 3). All pK_i values were derived from binding assays performed with cloned receptors unless otherwise specified. As can be seen, chlorprothixene, chlorpromazine, amoxapine, thioridazine, loxapine, clothiapine, fluphenazine, clorotepine (−) and perphenazine all had high affinities for the 5-HT_6 receptor (K_i < 20 nM). Pimozide, thiothixene, trifluoperazine and acetophenazine had intermediate affinities (K_i < 100 nM); mesoridazine, molindone and spiperone had low affinities (K_i < 1100 nM). Haloperidol, trifluoperidol, metoclopramide, rimcazole, remoxipride, raclopride and penfluridol did not significantly inhibit 5-HT_6 radioligand binding (K_i > 5000 nM).

The affinities of these drugs for the 5-HT_7 receptors differed in several respects. Pimozide (fig. 4, table 1) had the highest affinity of all the typical antipsychotic drugs tested (K_i = 0.5 nM); acetophenazine, chlorprothixene, fluphenazine, thiothixene and spiperone all had affinities less than 20 nM. Amoxapine, chlorpromazine, clothiapine, loxapine, perphenazine, thioridazine, trifluoperazine and trifluoperidol had intermediate affinities (K_is < 150 nM); haloperidol, molindone and prochlorperazine had low affinities (K_is < 1000 nM). Rimcazole, remoxipride, raclopride and penfluridol all had essentially no affinity for the 5-HT_7 receptors (K_is > 5000 nM).

Binding of atypical antipsychotic drugs to 5-HT_6 and 5-HT_7 receptors. We also evaluated the affinities of many putative atypical antipsychotic drugs for the cloned 5-HT_6 and 5-HT_7 receptors. The rationale for considering all the following compounds, with the exception of clorotepine, atypical was previously given (Meltzer *et al.*, 1989b; Roth *et al.*, 1992): clozapine, fluperlapine, rilapine, perlapine, tiospirone, olanzepine, risperidone, clorotepine (+), zotepine, amperozide, melperone and tenilapine. Bogeso *et al.* (1991) suggest that clorotepine (+) behaves atypically, although clorotepine (−) has a typical profile. For the 5-HT_6 receptor, zotepine, clorotepine (+ and −), clozapine, rilapine, olanzepine, tiospirone and flu-

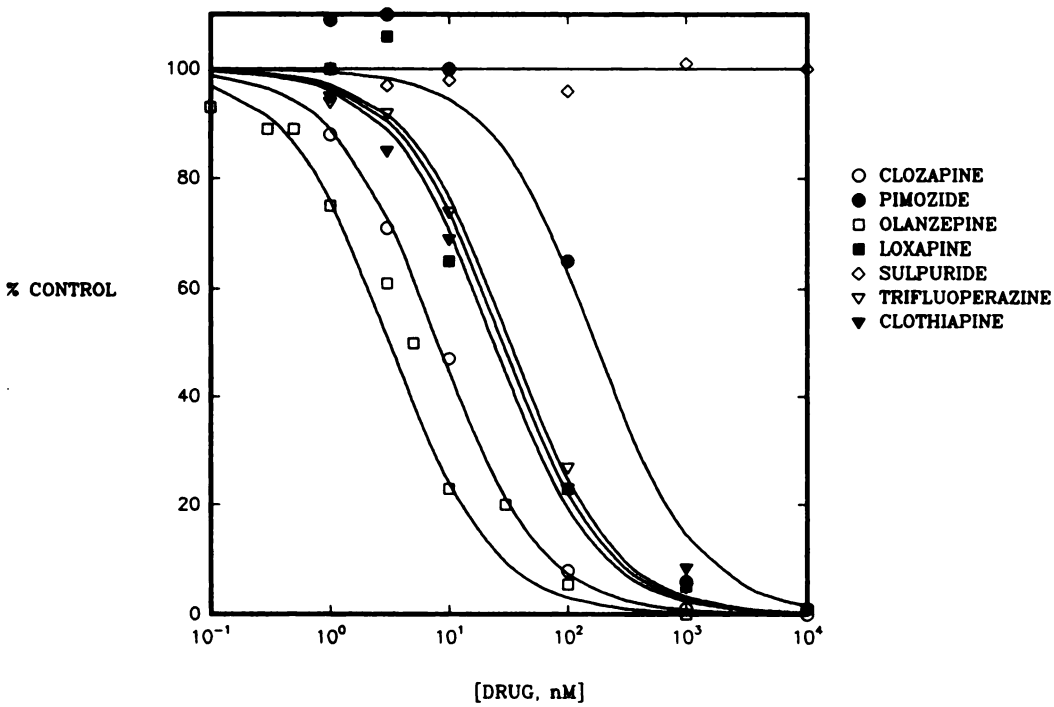


Fig. 3. Inhibition of [^{3}H]-LSD binding to 5-HT_6 receptors by various typical and atypical antipsychotic drugs. Shown are typical competition binding isotherms for [^{3}H]-LSD bound to 5-HT_6 receptors. The data represent the mean percent of the maximum specific binding (defined with 10 μM clozapine); the lines were drawn by the computer by using parameter estimates from the LIGAND program.

TABLE 1

Binding characteristics of 5-HT$_6$ and 5-HT$_7$ receptors

Data represent mean ± S.D. of computer-derived K_i values from LIGAND program for n = 2 to 4 separate experiments.

Drug	5-HT$_6$ K_i (pK$_i$)	5-HT$_7$ K_i (pK$_i$)
Phenothiazines		
Chlorpromazine (T)[a]	4 ± 1.4 (8.35)	21 ± 7 (7.6)
Thioridazine (T)	6.6 ± 1.7 (8.18)	70 ± 48 (7.15)
Fluphenazine (T)	17 ± 3.3 (7.77)	8 ± 5 (8.1)
Perphenazine (T)	17 ± 2.9 (7.77)	23 ± 7 (7.6)
Trifluoperazine (T)	66 ± 48 (7.18)	80 ± 13 (7.1)
Acetophenazine (T)	72 ± 48 (7.14)	2.4 ± 1 (8.62)
Prochlorperazine (T)	148 ± 92 (6.83)	196 ± 80 (6.7)
Mesoridazine (T)	159 ± 53 (6.8)	73 ± 40 (7.14)
Thioxanthenes		
Chlorprothixene (T)	3 ± 0.6 (8.52)	5.6 ± 1 (8.25)
Thiothixene (T)	45 ± 2.2 (7.35)	12 ± 1 (7.9)
Heterocyclic Compounds		
Zotepine (A)[b]	1.17 ± 0.4 (8.93)	1.78 ± 0.6 (8.75)
Clorotepine (+) (A)	0.41 ± 0.2 (9.38)	0.38 ± 0.05 (9.42)
Clorotepine (−) (T)	17.7 ± 5 (7.75)	1.3 ± 0.41 (8.89)
Clozapine (A)	4 ± 0.7 (8.40)	6.3 ± 3.7 (8.2)
Amoxapine (T)	6 ± 0.6 (8.22)	40.6 ± 25 (7.39)
Loxapine (T)	15 ± 1.5 (7.82)	43 ± 25 (7.37)
Clothiapine (T)	16 ± 6 (7.80)	4.3 ± 2.6 (8.37)
Fluperlapine (A)	16.5 ± 10 (7.78)	4.6 ± 2 (8.34)
Perlapine (A)	70 ± 21 (7.15)	28 ± 3 (7.55)
Butyrophenones		
Melperone (A)	1254 ± 359 (5.9)	578 ± 135 (6.24)
Spiperone (T)	1595 ± 842 (5.80)	9.9 ± 5.7 (8.00)
Trifluorperidol (T)	>5000 (<5.3)	63 ± 31 (7.2)
Haloperidol (T)	>5000 (<5.3)	263 ± 41 (6.58)
Diphenylbutylpiperidine		
Pimozide (T)	71 ± 30 (7.15)	0.5 ± 0.05 (9.3)
Penfluridol (T)	>5000 (<5.3)	>5000 (<5.3)
Diphenylbutylpiperazine		
Amperozide (A)	67 ± 12 (7.2)	549 ± 120 (6.3)
Miscellaneous Compounds		
Olanzepine (A)	2.5 ± 0.9 (8.6)	104 ± 12 (6.98)
Rilapine (A)	6.95 ± 38 (8.2)	2.8 ± 1.7 (8.6)
N-Desmethylclozapine (I)	9.4 ± 7 (8.02)	14.9 (7.8)
Tiospirone (A)	74 ± 52 (7.13)	0.64 ± 0.06 (9.19)
Risperidone (A)	425 ± 59 (6.37)	1.39 ± 1 (8.86)
MDL 100907 (A)	>5000	ND[d]
Sulpiride (T)	>5000	>5000
Rimcazole (T)	>5000	>5000
Raclopride (T)	>5000	>5000
Remoxipride	>5000	>5000
Molindone (T)	>5000	265 ± 36 (6.6)
Metoclopramide	>5000	ND
Clozapine-N-oxide (I)[c]	>5000	>5000
Chlorpromazine-N-Oxide (I)	>5000	>5000

[a] Typical.
[b] Atypical.
[c] Inactive.
[d] Not done.

TABLE 2

Binding of typical antipsychotic agents to cloned serotonin receptors

Shown are pKi values for cloned 5-HT1c, 5-HT2, 5-HT$_6$ and 5-HT$_7$ receptors except as otherside noted.

Drug	pK$_i$				
	5-HT$_6$	5-HT$_7$	5-HT$_2$	t-HT$_{1C}$	D2
Chlorprothixene	8.5	8.3	9.4[a]	ND[d]	ND
Chlorpromazine	8.4	7.6	8.6	7.6	8.9[b]
Amoxapine	8.2	7.4	9.0	8.7	ND
Thioridazine	8.2	7.2	8.2[a]	7.1	8.1[a]
Loxapine	7.8	7.4	8.7[a]	8.0	8.1[a]
Clothiapine	7.8	8.37	9.1	ND	ND
Fluphenazine	7.8	8.1	8.7	6.2	9.3[c]
Clorotepine (−)	7.8	8.6	ND	ND	7.9[a]
Perphenazine	7.8	7.6	8.6[a]	7.3	9.2[a]
Thiothixene	7.4	7.9	7.3[a]	5.8	9.2[a]
Trifluperazine	7.2	7.1	8.4[a]	7.0	9.7[c]
Pimozide	7.2	9.3	8.1[a]	<5	8.6[c]
Acetophenazine	7.1	8.6	ND	6.0	ND
Prochlorperazine	6.8	6.7	8.2[a]	7.1	8.3[a]
Mesoridazone	6.8	7.1	8.2	7.1	8.1[a]
Molindone	<5.3	6.6	6.3	ND	7.8[b]
Spiperone	5.8	8.0	9.48	6.01	10.2[c]
Haloperidol	<5.3	6.6	7.8	5.6	9.3[c]
Trifluperidol	<5.3	7.3	ND	ND	ND
Rimcazole	<5.3	<5.3	<5.3	ND	ND
Raclopride	<5.3	<5.3	<5.3	<5.3	8.7[c]
Penfluridol	<5.3	<5.3	7.2	ND	ND
Metoclopramide	<5.3	ND	ND	ND	ND

[a] Meltzer *et al.*, 1989 (rat cortex, not cloned).
[b] Malmberg *et al.*, 1993 (cloned, expressed in Ltk− cells).
[c] Van Tol *et al.*, 1991 (cloned, expressed in Ltk− cells).
[d] Not done.

TABLE 3

Binding of atypical antipsychotic agents to cloned serotonin receptors

Shown are pK$_i$ values obtained from cloned 5-HT$_{1C}$, 5-HT$_2$, 5-HT$_6$ and t-HT$_7$ receptors except as otherwise noted.

Drug	pK$_i$				
	5-HT$_6$	5-HT$_7$	5-HT$_{1C}$	5-HT$_2$	D2
Clorotepine (+)	9.4	9.4	ND[d]	ND	8.8[b]
Zotepine	9.0	8.8	ND	9.2[a]	9.0[a]
Olanzepine	8.6	7.0	ND	ND	ND
Clozapine	8.4	8.2	8.2	8.4	7.25[b]
Rilapine	8.2	8.6	7.6	9.2	7.4[a]
Fluperlapine	7.8	8.3	7.7	8.2	6.5[a]
Tiospirone	7.6	9.2	8.0	10.2	9.3[c]
Perlapine	7.2	7.6	ND	7.9[a]	6.3[a]
Amperozide	7.2	6.3	5.9	7.9	6.3[c]
Tenilapine	7.0	7.3	7.2	7.4	5.8[a]
Risperidone	6.4	8.9	7.5	9.6	8.8[c]
Melperone	5.9	6.3	5.9	7.1	6.7[a]

[a] Meltzer *et al.*, 1989 (rat striatum).
[b] Van Tol *et al.*, 1991 (cloned, Ltk− cells).
[c] Malmberg *et al.*, 1993 (cloned, Ltk− cells).
[d] Not done.

perlapine all had high affinities; amperozide, perlapine and tenilapine had intermediate affinities. Risperidone and melperone had weak affinities; the putative antipsychotic drug MDL 100907 and the pharmacologically inactive metabolite of clozapine, clozapine-N-oxide, had no affinity for the 5-HT$_6$ receptor.

For the 5-HT$_7$ receptor, compounds with structures similar to that of clozapine (fluperlapine and perlapine) had relatively high affinities, as did risperidone, zotepine, clorotepine (±) and tiospirone (tables 1–3). The major metabolite of clozapine, N-desmethylclozapine, had higher affinity for the 5-HT$_6$ than for the 5-HT$_7$ receptor. Interestingly, risperidone had a 303-fold higher affinity for the 5-HT$_7$ than for the 5-HT$_6$ receptor; olanzepine preferred the 5-HT$_6$ receptor by 40-fold (table 1). However, most atypical compounds had similar affinities for both 5-HT$_6$ and 5-HT$_7$ receptors.

The possibility that the relative affinity of atypical antipsychotic drugs for the 5-HT$_6$ and 5-HT$_7$ receptors compared with the D2 receptor distinguishes these compounds from typical antipsychotics was also examined because the previously reported 5-HT$_2$/D2 ratio was a sensitive index of atypicality (Meltzer *et al.*, 1989b). To do this, pK$_i$ values were used (tables

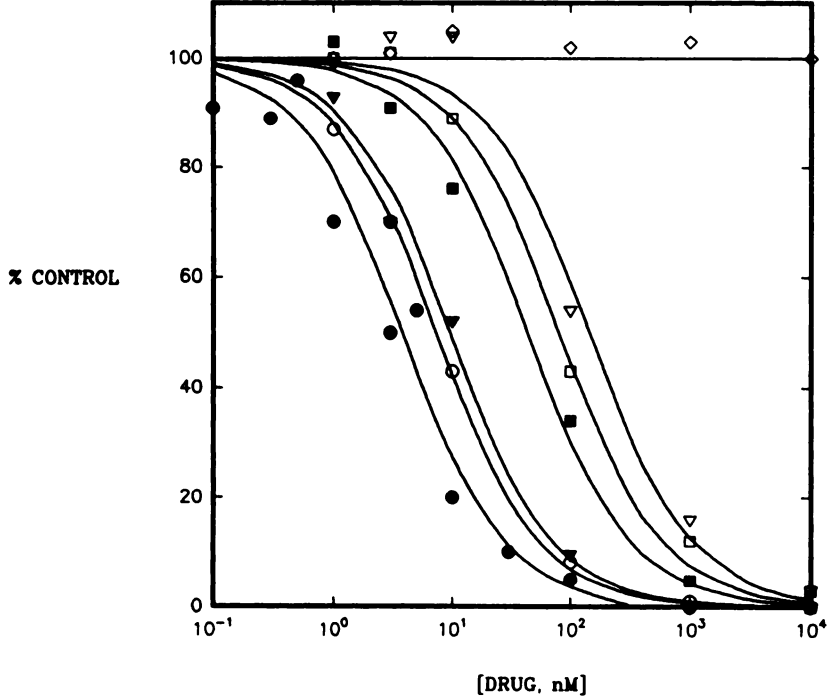


Fig. 4. Inhibition of [^{3}H]-LSD binding to 5-HT$_7$ receptors by various typical and atypical antipsychotic drugs. Shown are typical competition binding isotherms for [^{3}H]-LSD bound to 5-HT$_7$ receptors. The data represent the mean percent of maximum specific binding (defined with 10 μM clozapine); the lines were drawn by the computer by using parameter estimates from the LIGAND program.

2 and 3). The differences in the pK$_i$ values for the 5-HT$_6$ and D2, for the 5-HT$_7$ and D2 and for 5-HT$_2$ and D2 receptors were significantly greater for atypical than typical antipsychotic drugs ($P < .001$, data not shown). Stepwise discriminant function revealed that the pK$_i$ values for the D2 receptor contributed most to the discrimination, followed by the 5-HT$_2$ (average squared canonical correlation with D2 alone = 0.61; with 5-HT$_2$ added, 0.71). The stepwise discrimination did not find significant improvement in the prediction by adding 5-HT$_6$ or 5-HT$_7$ pK$_i$ values.

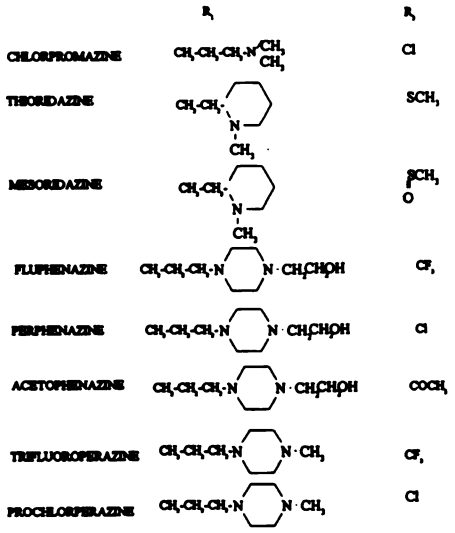
Discussion

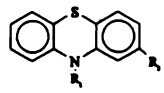
The major finding of this article was that a large number of both typical and atypical antipsychotic drugs have high affinities for the cloned rat 5-HT$_6$ and 5-HT$_7$ receptors. Clozapine and fluperlapine, both of which have been shown clinically to function as atypical antipsychotic agents (Korsgaard *et al.*, 1984; Woggon *et al.*, 1984; Woggon *et al.*, 1986; Kane *et al.*, 1988), had high affinities for both 5-HT$_6$ and 5-HT$_7$ receptors. These results suggest that a functional interaction with 5-HT$_6$ and/or 5-HT$_7$ receptors may contribute to the unique effects of some, but not all, atypical antipsychotic drugs. However, the ability of drugs to bind to 5-HT$_6$ and/or 5-HT$_7$ receptors does not appear to be essential for producing an antipsychotic drug that has little adverse effect on extrapyramidal function, *e.g.*, melperone.

Previous studies demonstrated that atypical antipsychotic drugs bind to a large number of neurotransmitter receptors, including dopaminergic (Meltzer *et al.*, 1989b), adrenergic (Cohen and Lipinski, 1986), cholinergic (Bolden *et al.*, 1992) and serotonergic receptors (Meltzer and Nash, 1991). Studies of the importance of 5-HT receptors for an atypical antipsychotic profile have concentrated on the 5-HT$_2$ receptor and, to a lesser extent, the 5-HT$_{1C}$ (Roth *et al.*,1992) and 5-HT$_3$ and 5-HT$_4$ receptors (Meltzer and Nash, 1991). Thus, Meltzer *et al.* (1989b) demonstrated that atypical antipsychotic drugs, as a group, have a ratio of 5-HT$_2$/D2 K_i values greater than 10; typical antipsychotic drugs have 5-HT$_2$/D2 K_i values less than 10. Since those studies were published, 13 5-HT receptor subtypes have been cloned: 5-HT$_{1A}$ (Kobilka *et al.*, 1987), 5-HT$_{1D\alpha}$ (Hamblin and Metcalf, 1991), 5-HT$_{1D\beta}$ (Voight *et al.*, 1991), 5-HT$_{1C}$ (Julius *et al.*, 1988), 5-HT$_{1E\alpha}$ and 5-HT$_{1E\beta}$ (Amlaiky *et al.*, 1992; McAllister *et al.*, 1992; Zgombick *et al.*, 1992; Lovenberg *et al.*, 1993), 5-HT$_2$ (Pritchett *et al.*, 1988), 5-HT$_{2F}$ (Kursar *et al.*, 1992), 5-HT$_3$ (Maricq *et al.*, 1991), 5-HT$_{5A}$ and 5-HT$_{5B}$ (Matthes *et al.*, 1993), 5-HT$_6$ (Monsma *et al.*, 1993) and 5-HT$_7$ (Shen *et al.*, 1993). The present studies represent an extension of our prior studies. Previously, we examined the 5-HT$_{1C}$ receptor and found that the spectrum of drug binding did not favor a hypothesis that implicated the 5-HT$_{1C}$ receptor in antipsychotic drug actions (Roth *et al.*, 1991). We now find that most, but not all, atypical antipsychotic drugs, and selected typical antipsychotic drugs, bind strongly to 5-HT$_6$ and 5-HT$_7$ receptors.

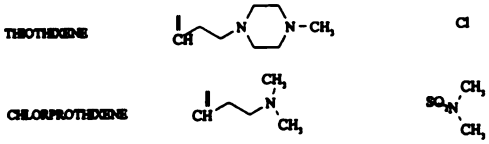
Monsma *et al.* (1993) reported the cloning and preliminary pharmacological characterization of the 5-HT$_6$ receptors. These authors found that many psychoactive compounds with tricyclic and/or heterocyclic structures had high affinities for the 5-HT$_6$ receptor. Interestingly, these authors reported that the 5-HT$_6$ receptor was highly enriched in striatum and certain limbic regions. Our results suggest that many atypical antipsychotic drugs can bind with high affinity to the 5-HT$_6$ receptor. Although we do not know the physiological relevance of this high-affinity binding, it is conceivable that the lack of extrapyramidal side effects of clozapine and related compounds could be the result in part of an action at 5-HT$_6$ receptors in the striatum. In this regard, it is interesting to note that risperidone, which does not have high affinity for the 5-HT$_6$ receptor, can cause extrapyramidal side effects in humans at high doses (Chouinard *et al.*, 1993). Consistent with this, thioridazine, which does not cause catalepsy and appears to have other atypical features in rats (Merchant and Dorsa, 1993), has a relatively high affinity for the 5-HT$_6$ receptor.

PHENOTHIAZINES





THIOXANTHENES



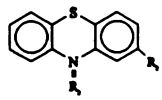
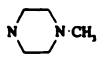
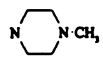
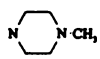
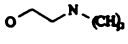
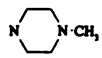
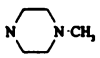
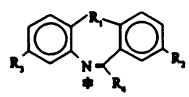


Fig. 5. Structures of phenothiazines and heterocyclic compounds. *For zotepine and clorotepine (octoclothepin), N = C. For clorotepine (±), the 7-membered ring is fully saturated.

CLOZAPINE-LIKE COMPOUNDS

	R1	R2	R3	R4
CLOZAPINE	N	H	Cl	
FLUPERLAPINE	C	H	F	
PERLAPINE	C	H	H	
LOXAPINE	O	Cl	H	
AMOXAPINE	O	Cl	H	
ZOTEPINE	S	Cl	H	
CLOTHIAPINE	S	Cl	H	
OCTOCLOTHEPIN(+/-)	S	Cl	H	

*FOR ZOTEPINE N=C FOR OCTOCLOTHEPIN N=C AND NO DOUBLE BOND



Shen *et al.* (1993) cloned another 5-HT receptor that they named 5-HT_7. As we report here, the 5-HT_7 receptor has high affinity for pimozide (table 1). Pimozide is a somewhat novel antipsychotic agent that is effective in the treatment of Tourette's syndrome, certain cases of obsessive-compulsive disorder and tics (Cohen *et al.*, 1992). It is conceivable that some of the unique actions of pimozide are dependent in part on an action at the 5-HT_7 receptor.

We found no mean differences between the pK_i values of typical and atypical antipsychotic drugs for 5-HT_6 and 5-HT_7 receptors (not shown). This result was similar to our previous finding with regard to the 5-HT_2 receptor (Meltzer *et al.*, 1989b). There was also no clear pattern of greater affinity for the 5-HT_6 or 5-HT_7 receptors for either class of drugs. Because we reported that the ratio of the K_i values for 5-HT_2 and D2 receptor binding significantly differentiated atypical and typical antipsychotic drugs (Meltzer *et al.*, 1989b), a similar approach was tried by using 5-HT_6 and 5-HT_7 ratios. We found no contribution for the 5-HT_6 and 5-HT_7 receptors for the group as a whole. However, this analysis does not rule out the possibility that, for specific drugs in which the 5-HT_6 or 5-HT_7 affinities are high, occupancy of 5-HT_6 or 5-HT_7 receptors could contribute to certain therapeutic actions. It is interesting that the affinities for all drugs for the 5-HT_2 receptor, with the exception of thiothixene and clozapine, was equal to or greater than that for the 5-HT_6 and 5-HT_7 receptors.

One of the difficulties with a mere statistical analysis of the findings by a stepwise discriminant function analysis is that only one aspect of an atypical antipsychotic drug's actions is considered (*i.e.*, lack of extrapyramidal side effects). Clozapine, which is the prototypic atypical antipsychotic drug, has unique actions, which include its effectiveness in treatment-resistant schizophrenia, its apparent lack of induction of tardive dyskinesia and its ability to suppress symptoms of tardive dyskinesia (Kane *et al.*, 1988; Lieberman *et al.*, 1991; Meltzer and Luchins, 1984). No currently available antipsychotic drug has these unique actions, although it is important to remember that none of the drugs listed as atypical antipsychotics has been used in large-scale trials for treatment-resistant schizophrenia or tardive dyskinesia. At the present time, we do not know if clozapine's high affinities for the 5-HT_6 and/or 5-HT_7 receptors is essential for its unique actions. When more clinical information is available on the effectiveness of the drugs listed in table 2, we may be better able to rule in or out a role for the 5-HT_6 and 5-HT_7 receptors for the actions of clozapine and similar compounds.

In terms of structure-activity relationships (fig. 5), we are able to reach several conclusions in regard to the tricyclic and heterocyclic molecules and their ability to recognize 5-HT_6 receptors. For phenothiazines, an aldehyde in the R2 position greatly decreased affinity for the 5-HT_6 receptor. The nature of the R1 substituent had little effect with the derivatives tested, except in the case of chlorpromazine N-oxide, which was devoid of affinity for the 5-HT_6 and 5-HT_7 receptors. For the 5-HT_7 receptors, in general, the bulkier the substitution at the R2 position is, the higher the affinity is; this was particularly clear with acetophenazine > fluphenazine > perphenazine and trifluoperazine > prochlorperazine. Similar to the 5-HT_6 receptor, the nature of the R2 substituent had little effect on binding affinity with the exception again, of chlorpromazine N-oxide.

For heterocyclic molecules, a halogen at either the R2 or R3 position was associated with high affinity. This is most clearly seen by comparing fluperlapine and perlapine. This relationship did not hold consistently with the 5-HT_7 receptors, in which, for instance, loxapine and amoxapine both had lower affinities than did perlapine. The nature of the R4 substituent had apparently little effect on the affinities for both the 5-HT_6 and 5-HT_7 receptors. Also, the presence of a nitrogen, carbon, oxygen or sulfur at the R1 position had no detectible effect on the affinity for the 5-HT_6 receptor (compare loxapine and clothiapine). For the 5-HT_7 receptor, however, an oxygen at the R1 position was generally associated with lower affinity than was a sulfur (compare clothiapine and loxapine). Demethylation of the ring nitrogen at R4 had no large effect on the affinity for either the 5-HT_6 or 5-HT_7 receptors.

In general, butyrophenones had low affinities for the 5-HT_6 receptor. For the 5-HT_7 receptor, the nature of the substituent had great effects on the affinity. In general, the larger the group is, the higher the apparent affinity is. Thus, for the butyrophenone series, we found the following relationship for the 5-HT_7 receptor: spiperone > trifluoperidol > haloperidol > melperone. The presence of nitrogens greatly increased the affinity for the 5-HT_7 receptor; this was seen for both the butyrophenones and the diphenylbutypiperidines. Taken together, these findings demonstrate that the 5-HT_6 and 5-HT_7 receptors, although they both have a high affinity for many antipsychotic agents, have distinct structure-activity relationships. These differences suggest that 5-HT_6 and/or 5-HT_7-subtype selective agents could be synthesized.

In conclusion, we discovered that two recently cloned 5-HT receptors, 5-HT_6 and 5-HT_7, possessed high affinities for clozapine and related atypical antipsychotic agents. The high affinity of selected typical and atypical antipsychotic agents for these newly cloned 5-HT receptors may be important for mediating the unique actions of certain antipsychotic drugs. In particular, the high affinity for the 5-HT_6 receptor, in view of its concentration in the striatum, suggests that 5-HT_6-active agents could be important for certain motor effects of typical and atypical antipsychotic agents. The availability of 5-HT_6 and/or 5-HT_7 receptor selective agents will shed light on the physiological role of these interesting receptors.

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