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Molecular structural basis of ligand selectivity for 5-HT_2 versus 5-HT_{1C} cortical receptors

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Summary. A molecular structural criterion of ligand selectivity for the 5-HT_2 versus 5-HT_{1C} receptor was hypothesized on the basis of radioligand binding data. Despite the large number of compounds which have been tested at both receptors, analysis of published data led to the identification of only five agents which are greater than 10-fold selective for the 5-HT_2 versus the 5-HT_{1C} receptor. Comparison of the two-dimensional structures revealed that, although these five compounds represent three distinct structural classes, they share a common structural feature located in the region hypothesized to be involved in receptor binding: a carbonyl or carboxyl oxygen interposed spatially between an aromatic ring and nitrogen atom. This structural feature was used to predict the relative selectivity of compounds that had not previously been analyzed at both the 5-HT_2 and 5-HT_{1C} receptors.

All six drugs tested which contain the identified reactive carbonyl or carboxyl group were found to be selective for the 5-HT_2 versus the 5-HT_{1C} receptor with selectivity ratios ranging from 26 to 380. By contrast, three agents which are structurally similar but do not contain the reactive carbonyl or carboxyl group displayed equally high affinity for both receptor binding sites. Since the physiological roles of the 5-HT_2 and 5-HT_{1C} receptor are markedly different, it would be of potential clinical and scientific value to utilize this molecular structural feature to further identify chemical compounds which would selectively interact with only one of the two receptors.

Key words: 5-HT_2 receptor – 5-HT_{1C} receptor – 5-HT ligand selectivity

Introduction

The 5-HT_{1C} and 5-HT_2 receptors are similar in terms of their structure, biochemistry and pharmacology. Both receptors have been cloned and sequenced and have been shown to contain seven putative transmembrane-spanning segments which display 80% homology (Julius et al. 1988; Pritchett et al. 1988). Both receptors are linked to G-proteins and use phosphatidylinositol hydrolysis as a second-messenger system for signal transduction (Conn and Sanders-Bush 1984; Conn et al. 1986). Furthermore, the two receptor subtypes share an extremely similar pharmacological profile for numerous classes of compounds (Pazos et al. 1984a).

Nonetheless, the distribution and physiological role of the 5-HT_{1C} and 5-HT_2 receptor subtypes are markedly different. 5-HT_{1C} receptors are located most densely in the choroid plexus and have been hypothesized to play a role in cerebrospinal fluid production, whereas 5-HT_2 receptors are located most densely in cortical layers 4 and 5 and are thought to play a role in the pathophysiology of migraine, anxiety disorders and depression (Lindvall-Axelsson et al. 1988; Peroutka et al. 1989). Therefore, it would be of potential clinical and scientific value to develop chemical compounds which would selectively interact with only one of these two receptors.

To date, only a few drugs have been reported to interact differentially with 5-HT_{1C} and 5-HT_2 receptors (Pazos et al. 1984a). Since the 5-HT_2 receptor is implicated in a variety of CNS disorders, the goal of the present study was to determine if a common molecular structural feature(s) could be identified in the few drugs known to display higher affinity for 5-HT_2 versus 5-HT_{1C} receptors. If such features were identified, the resulting hypothesis could be tested by determining the 5-HT_{1C} and 5-HT_2 receptor affinities of previously unanalyzed compounds.

Methods

Radioligand binding studies were performed according to the methods of Pazos et al. (1984a) and McKenna and Peroutka (1989) Porcine and

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bovine brains were obtained from Pel-Freez (Rogers, Ark., USA) and the cortex was dissected and frozen at −70 °C until use. Tissues were homogenized in 50 mmol/l Tris-HCl buffer (pH 7.7 at 25 °C) and then centrifuged at 39,000×g for 10 min. The supernatant was discarded and the pellet was resuspended in the same volume of Tris-HCl buffer and incubated at 37 °C for 10 min prior to a second centrifugation. The final pellet was resuspended in 80 volumes of 50 mmol/l buffer containing 10 µmol/l pargyline, 4 mmol/l calcium chloride and 0.1% ascorbic acid. The membrane suspensions were immediately used in the binding assay.

Radioligand binding studies consisted of 0.1 ml ^{3}H-radioligand (0.2–0.5 nmol/l ^{3}H-ketanserin; 0.7–1.2 nmol/l ^{3}H-mesulergine), 0.1 ml buffer or displacing drug and 0.8 ml tissue homogenate. Nonspecific binding was measured in the presence of 10^{-5} mol/l 5-HT for 5-HT$_{1C}$ sites and 10^{-6} mol/l cinanserin for 5-HT$_2$ sites with specific binding defined as the total binding minus the nonspecific binding. Following incubation at 25 °C for 30 min, the assays were rapidly filtered under vacuum through No. 32 glass fiber filters (Schleicher and Schuell, Keene, N.H., USA) with two 5 ml washes using 50 mmol/l Tris-HCl buffer. Radioactivity retained on the filters was measured by liquid scintillation spectroscopy in 2.5 ml of scintillation fluid (Biosafe II; Research Products International Corp., Mount Prospect, Ill., USA) at 61% efficiency. All experiments were performed in triplicate and repeated 3–7 times. The inhibitory concentration$_{50}$ (IC_{50}) value was calculated by log-logit analysis and the K_i values calculated by the formula $K_i = IC_{50}/(1+([ligand]/K_d))$, where $K_d = 1.7$ nmol/l for ^{3}H-mesulergine in porcine cortex (Pazos et al. 1984a) and $K_d = 1.2$ nmol/l for ^{3}H-ketanserin in bovine cortex (McKenna and Peroutka 1989). Bovine cortex was used to study 5-HT$_2$ receptor affinity since previous studies (Pierce and Peroutka 1989a) have shown this receptor population to be homogeneous. In addition to the classically defined 5-HT$_2$ receptors, which correspond to the sites in bovine brain, brain tissue from rat and human contain a subpopulation of high affinity sites not yet identified as separate "sites" versus "states" of 5-HT$_2$ receptors, thereby making receptor binding results with these tissues more difficult to interpret. IC_{50} and K_i values are reported as average ± standard error of the mean (SEM).

All drugs were diluted and dissolved in assay buffer. Radiolabeled drugs were obtained from Dupont, New England Nuclear (Boston, Mass., USA) (^{3}H-ketanserin, 73.5 Ci/mmol) and Amersham (Chicago, Ill., USA) (^{3}H-mesulergine, 79.0 Ci/mmol). Drugs were obtained from the following sources: droperidol, ketanserin tartrate, pipamperone, pirenperone, ritanserin and spiperone were gifts from Janssen Pharmaceutica (Piscataway, N.J., USA); cloroperone, MDL 11939, MDL 26508 and MDL 28133 were gifts from Merrell Dow Pharmaceuticals (Cincinnati, Ohio, USA); tiospirone HCl was a gift from Bristol-Myers Co. (Evansville, Ind., USA); 5-HT creatinine sulfate (Sigma Chemical Co., St. Louis, Mo., USA); cinanserin HCl (E.R. Squibb & Sons, Inc., Princeton, N.J., USA); haloperidol (McNeil Pharmaceutical, Springhouse, Pa., USA); trazodone HCl was a gift from R. Duman.

Results

Comparison of reported drug affinities for 5-HT$_{1C}$ and 5-HT$_2$ receptors

5-HT$_{1C}$ and 5-HT$_2$ binding affinity data for various classes of compounds were compiled from the literature and drug affinities reported in two or more publications are shown graphed in Fig. 1, with the corresponding compounds listed in Table 1. The graphed data points can be placed into three major categories: (1) Drugs displaying at least 10-fold higher affinity for the 5-HT$_{1C}$ receptor; (2) Drugs with relatively equal affinity for 5-HT$_{1C}$ and 5-HT$_2$ receptors (between dotted lines on graph), shown with a straight-line fit of slope = 0.97 and correlation coefficient of r = 0.93; (3) Drugs displaying at least a 10-fold higher affinity for the 5-HT$_2$ receptor.

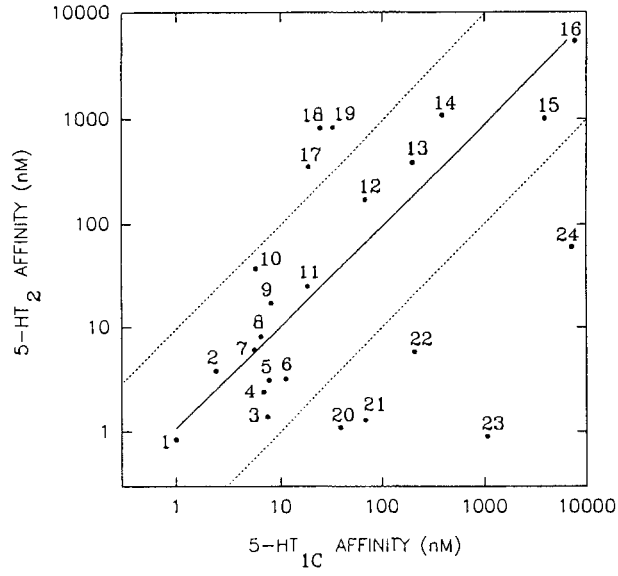

Fig. 1. Correlation of drug affinities for 5-HT$_2$ versus 5-HT$_{1C}$ receptors. Numbers shown correspond with the compound names listed in Table 1. Data shown are the log average of K_i values from at least two published reports in the literature. Following is a list of references from which the data were obtained: Creese and Snyder 1978; Leysen et al. 1978, 1982, 1986, 1988; Peroutka and Snyder 1979, 1980; Battaglia et al. 1983, 1984; Closse 1983; Hartig et al. 1983, 1985; Andorn et al. 1984; Kadan et al. 1984; Pazos et al. 1984a; Titeler et al. 1984, 1987, 1988; Glaser and Traber 1985; Hoyer et al. 1985, 1986, 1987, 1989; Peroutka 1985, 1988; Engel et al. 1986; Fuller et al. 1986; Glennon et al. 1986; Lyon et al. 1986, 1987, 1988; Markstein et al. 1986; Tricklebank et al. 1986; Yagaloff et al. 1986; Fozard et al. 1987; Hoffman et al. 1987; Neale et al. 1987; Roth et al. 1987; Wander et al. 1987; Wouters et al. 1987; Blackburn et al. 1988; Dudley et al. 1988; Hoyer 1988a, 1988b, 1989; Hoyer and Karpf 1988; Nicklaus et al. 1988; Sanders-Bush and Breeding 1988; Taylor et al. 1988; Elliott and Kent 1989; Hamik and Peroutka 1989; McKenna and Peroutka 1989; Pierce and Peroutka 1989b; Sadzot et al. 1989; Schoeffter and Hoyer 1989; Yamamoto and Kebabian 1989

5-HT$_{1C}$-selective agents

An interesting feature of the three agents that display 10-fold higher affinity for 5-HT$_{1C}$ is that they are all agonists in biochemical studies (Conn et al. 1986; Conn and Sanders-Bush 1987). In the present study, we chose not to pursue a structural analysis of this select group of agents since the receptor-ligand interaction of agonists is more complex (e.g. high affinity versus low affinity receptor states for agonists) than for competitive antagonist receptor-ligand interactions.

Comparison of two-dimensional structures for non-selective versus 5-HT$_2$-selective agents

It is the group of drugs displaying higher affinities for 5-HT$_2$ receptors relative to 5-HT$_{1C}$ receptors that were further analyzed in the present study. The five 5-HT$_2$-selective agents are all antagonists in both 5-HT$_{1C}$ and 5-HT$_2$ functional studies (Kendall and Nahorski 1985; Doyle et al. 1986; Roth et al. 1986; Sanders-Bush and

Breeding 1988; Hoyer et al. 1989). Shown in Fig. 2 are the core molecular structures of the drugs which are equipotent at 5-HT_{1C} and 5-HT_2 receptors. The aromatic ring(s) and nitrogen atom hypothesized to be key in receptor interaction are indicated by an asterisk (*) (Lloyd and Andrews 1986; Hibert et al. 1988; Schmidt and Peroutka 1989). In contrast, the molecular structures of the five drugs which are selective for 5-HT_2 relative to 5-HT_{1C} receptors are shown in Fig. 3. The hypothesized "receptor-docking" aromatic ring and nitrogen are also indicated by an asterisk (*). In this putative critical region of the molecule, however, there is a structural difference between the 5-HT_2-selective versus non-selective antagonist structures: a carbonyl oxygen (denoted by **) interposed spatially between the aromatic ring and the nitrogen atom.

Table 1. Compounds previously tested at both 5-HT_{1C} and 5-HT_2 receptors as reported in the literature

A. Drugs with relatively equal affinity for 5-HT_{1C} and 5-HT_2 receptors (graphed between dotted lines on Fig. 1)

1. Ritanserin
2. Mesulergine
3. Methiothepin
4. Pizotifen
5. d-Lysergic acid diethylamide (d-LSD)
6. Cyproheptadine
7. Mianserin
8. SCH 23390
9. LY 53857
10. 1-(1-naphthyl)-piperazine (1-NP)
11. 2,5-Dimethoxy-4-iodo-amphetamine (DOI)
12. m-Trifluoromethylphenyl-piperazine (TFMPP)
13. Quipazine
14. RU 24696
15. Buspirone
16. 8-Hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT)

B. Drugs displaying at least 10-fold higher affinity for 5-HT_{1C} receptors (graphed in Fig. 1)

17. m-Chlorophenylpiperazine (mCPP)
18. 5-Methoxytryptamine (5-MeOT)
19. 5-Hydroxytryptamine (5-HT)

C. Drugs displaying at least 10-fold higher affinity for 5-HT_2 receptors (graphed in Fig. 1)

20. Pirenpirone
21. Ketanserin
22. Cinanserin
23. Spiperone
24. Haloperidol

The 5-HT_{1C} and 5-HT_2 receptor affinities for the 5-HT_2-selective compounds were determined in our laboratory by drug competition studies performed as described in Methods. Corresponding K_i values and selectivity ratios (5-HT_{1C} K_i [nmol/l]/5-HT_2 K_i [nmol/l]) are listed for each compound in Table 2. The two butyrophenones, spiperone and haloperidol, display the highest degree of selectivity for the 5-HT_2 site with ratios of 990 and 190, respectively. The two benzoylpiperidinyl compounds, ketanserin and pirenperone, display selectivity for the 5-HT_2 receptor with ratios of 20 and 18, respectively. Cinanserin is the least selective for the 5-HT_2 receptor with a ratio of 15. These results are similar to published results from other laboratories as shown in Fig. 1.

Analysis of previously untested compounds with a potentially reactive carbonyl or carboxyl group at 5-HT_{1C} and 5-HT_2 receptor binding sites

In order to determine the possible role of the carbonyl oxygen in 5-HT_2 versus 5-HT_{1C} receptor selectivity, we test-

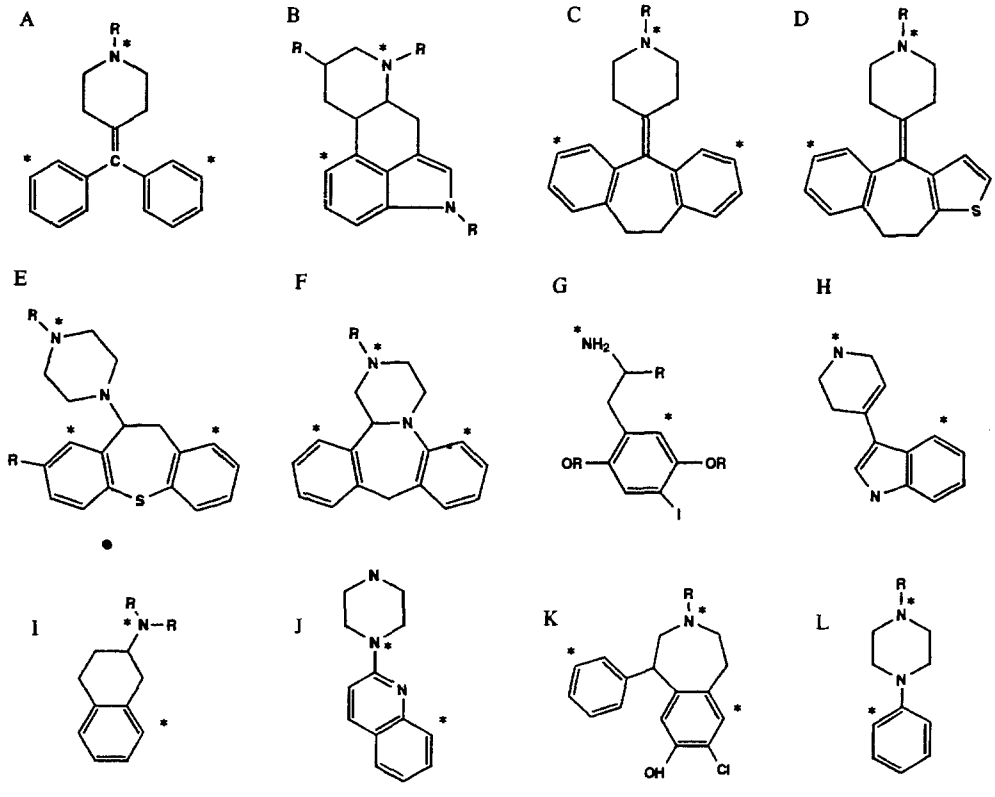

Fig. 2A–L. Core structures of various 5-HT_2/5-HT_{1C} non-selective drugs. The letters shown for each 2D structure correspond to the following compound names. **A** ritanserin; **B** mesulergine, d-LSD, LY 53857; **C** cyproheptadine; **D** pizotifen; **E** methiothepin; **F** mianserin; **G** DOI; **H** RU 24969; **I** 8-OH-DPAT; **J** quipazine; **K** SCH 23390; **L** TFMPP, 1-NP, buspirone. Note: Generalized structures only – not all atomic substitutions are shown

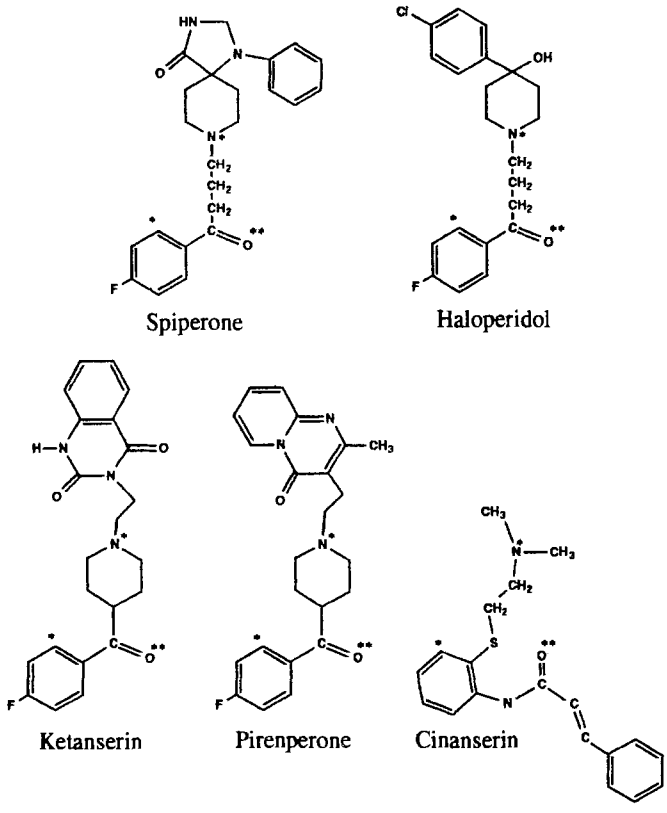

Fig. 3. Two-dimensional molecular structures of previously reported 5-HT_2 selective agents. Aromatic ring(s) and the nitrogen atom hypothesized to be involved in receptor-docking are denoted with (*) and the carbonyl oxygen is denoted with (**)

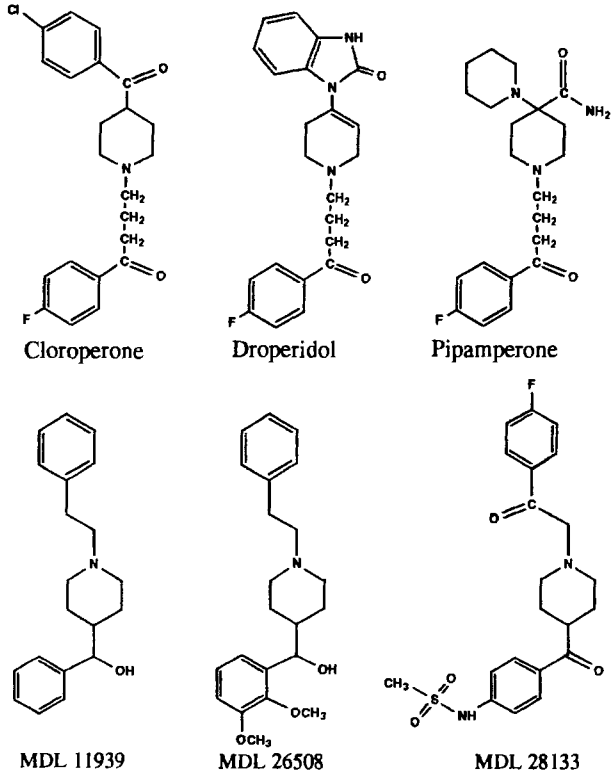

Fig. 4. Two-dimensional molecular structures of novel compounds containing reactive carbonyl or carboxyl group interposed between hypothesized receptor-docking aromatic ring and nitrogen atom

Table 2. Summary of drug affinities (K_i for 5-HT_{1C} and 5-HT_2 receptors)

Drug	5-HT_{1C} (nmol/l)	5-HT_2 (nmol/l)	Ratio (5-HT_{1C}/5-HT_2)
I. Previously reported 5-HT_2-selective agents			
Spiperone	960 ±200	0.97 ± 0.4	990
Haloperidol	2800 ±900	15 ±2	190
Ketanserin	49 ± 10	2.4 ±1	20
Pirenperone	21 ± 6	1.2 ±0.3	18
Cinanserin	56 ± 6	3.7 ±1	15
II. Novel compounds containing reactive carbonyl/carboxyl group			
Cloroperone	1700 ±500	4.5 ±1	380
Droperidol	110 ± 40	1.3 ±0.3	85
Pipamperone	90 ± 20	1.8 ±1	50
MDL 11939	500 ± 80	3.1 ±0.9	160
MDL 26508	170 ± 5	2.3 ±1	74
MDL 28133	420 ± 40	16 ±2	26
III. Similar compounds not containing reactive carbonyl/carboxyl group			
Ritanserin	6.3 ± 1	13 ±0.6	0.5
Tiospirone	8.5 ± 3	7.7 ±4	1.1
Trazodone	10 ±3	13 ±5	0.8

ed various compounds which contain a potentially reactive oxygen in this same location, either in a carbonyl or a carboxyl group, to determine affinities for 5-HT_{1C} and 5-HT_2 receptors. These six compounds, shown in Fig. 4, have not been tested previously at both 5-HT_{1C} and 5-HT_2 receptors, or have been reported only once in the literature (pipamperone; Leysen 1990).

The result of drug competition studies for the three butyrophenones – cloroperone, droperidol and pipamperone, at [^{3}H]-mesulergine-labelled 5-HT_{1C} receptors in porcine cortex and [^{3}H]-ketanserin-labelled 5-HT_2 receptors in bovine cortex are shown in Fig. 5 A and B, respectively. The K_i values and receptor selectivity ratios are summarized in Table 2. All three drugs possess a high degree of selectivity for 5-HT_2 versus 5-HT_{1C} receptors. Cloroperone is the most selective of the three butyrophenones, displaying a 380-fold selectivity for 5-HT_2 receptors. Droperidol displays an 85-fold selectivity for 5-HT_2 sites. Pipamperone is the least selective of the three drugs for 5-HT_2 receptors displaying a 50-fold selectivity for 5-HT_2 receptors versus 5-HT_{1C} sites.

The results of drug competition studies testing the carboxyl-containing agent MDL 11939, MDL 26508 and the carbonyl-containing agents MDL 28133 against [^{3}H]-mesulergine-labelled 5-HT_{1C} receptors in porcine cortex and [^{3}H]-ketanserin-labelled 5-HT_2 receptors in bovine cortex are shown graphed in Fig. 6 A and B, respectively, and summarized in Table 2. Again, all three compounds are highly selective for 5-HT_2 versus 5-HT_{1C} receptors. MDL 11939 displays the highest selectivity for 5-HT_2 receptors (160-fold) of the three MDL compounds. MDL 26508 displays a 74-fold selectivity for 5-HT_2 sites and MDL 28133 was the least selective of the new drugs tested, displaying a 26-fold selectivity for 5-HT_2 versus 5-HT_{1C} receptors.

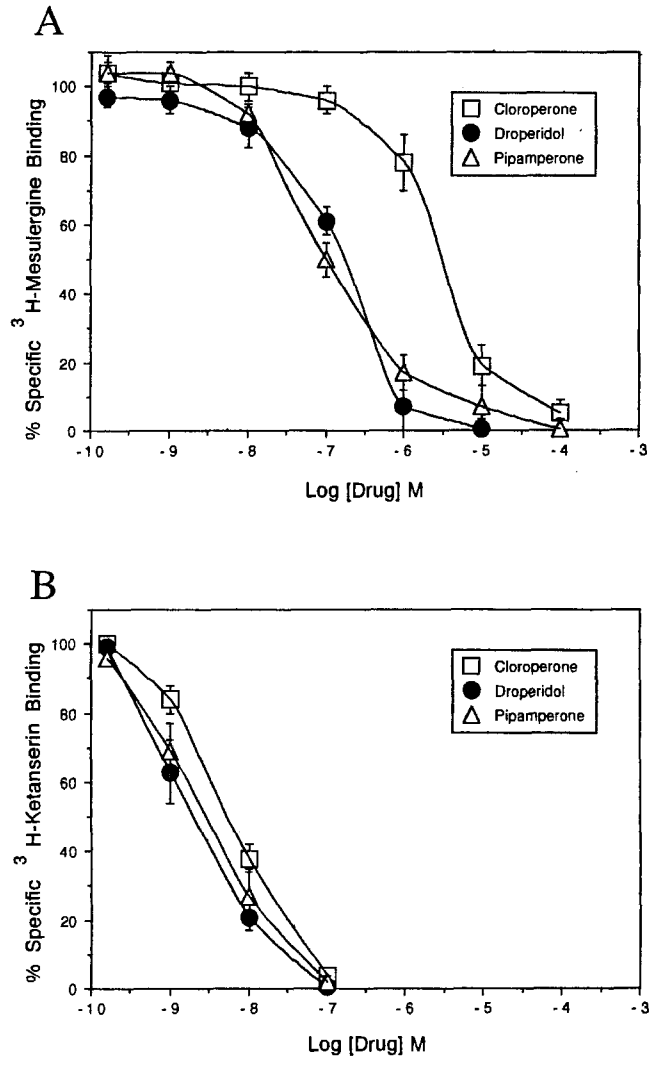

Fig. 5. Drug competition studies versus specific [^{3}H]-mesulergine-labelled 5-HT_{1C} receptors in porcine cortical membranes **(A)** and [^{3}H]-ketanserin-labelled 5-HT_2 receptors in bovine cortical membranes **(B)**. Radioligand studies were performed as described in Materials and methods, with nonspecific binding determined in the presence of 10^{-5} M 5-HT **(A)** and 10^{6} M cinanserin **(B)**. Data shown are the means ± SE of three to seven experiments using cloroperone (□), droperidol (●), and pipamperone (△)

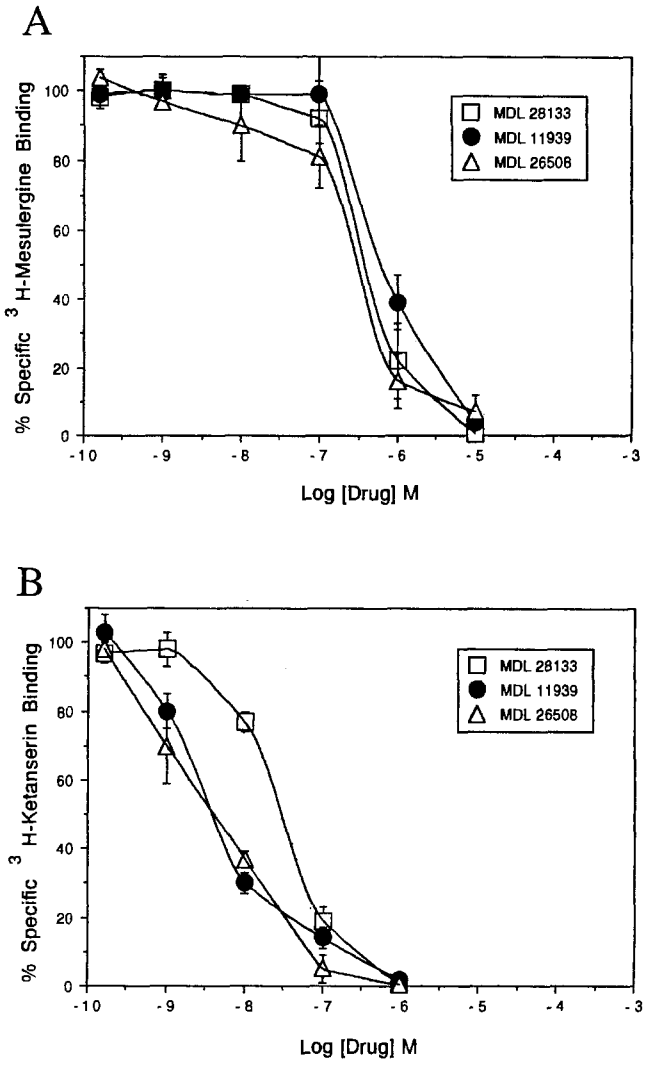

Fig. 6. Drug competition studies versus specific [^{3}H]-mesulergine-labelled 5-HT_{1C} receptors in porcine cortical membranes **(A)** and [^{3}H]-ketanserin-labelled 5-HT_2 receptors in bovine cortical membranes **(B)**. Radioligand studies were performed as described in Materials and methods, with non-specific binding determined in the presence of 10^{-5} M 5-HT **(A)** and 10^{-6} M cinanserin **(B)**. Data shown are the means ± SE of three to seven experiments using MDL 28133 (□), MDL 11939 (●), and MDL 26508 (△)

Analysis of similar compounds without a reactive carbonyl or carboxyl group at 5-HT_{1C} and 5-HT_2 receptor binding sites

The molecular structures of the wide variety of non-selective compounds shown in Fig. 2 do not contain a carbonyl or carboxyl group interposed between the key aromatic and nitrogen groups. Of particular interest is the drug ritanserin which contains a molecular structure quite similar to the 5-HT_2-selective benzoylpiperidinyl compounds, ketanserin and pirenperone, with the exception that ritanserin contains a phenyl group in place of the reactive carbonyl group (Fig. 7). In order to further test the hypothesis that the reactive oxygen located between the aromatic group and the key nitrogen atom plays a role in selective ligand affinity for 5-HT_2 receptors versus 5-HT_{1C} receptors, we chose to test ritanserin and two additional compounds, tiospirone and trazodone, which share a structure similar to the 5-HT_2-selective agents yet do not contain the specified carbonyl or carboxyl group. These last two compounds have not been tested previously at both 5-HT_{1C} and 5-HT_2 receptors. The molecular structures of these three compounds are shown in Fig. 7.

The results of drug competition studies testing ritanserin, tiospirone and trazodone against [^{3}H]-mesulergine-labelled 5-HT_{1C} receptors in porcine cortex and [^{3}H]-ketanserin-labelled 5-HT_2 receptors in bovine cortex are summarized in Table 2. None of the compounds showed selectivity for 5-HT_2 relative to 5-HT_{1C} receptors. In fact, ritanserin displays a 2-fold selectivity for 5-HT_{1C} receptors. Tiospirone and trazodone display equally high affinity for both 5-HT_2 and 5-HT_{1C} receptors.

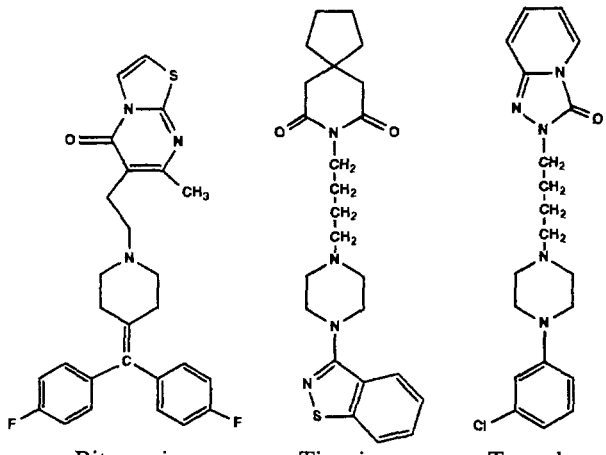

Fig. 7. Two-dimensional molecular structures of previously tested (ritanserin), and novel (tioperone, trazodone) compounds without a reactive carbonyl or carboxyl group interposed between receptor-docking aromatic ring(s) and nitrogen atom

Discussion

The major finding of the present study is that a reactive carbonyl or carboxyl group interposed between the aromatic ring and nitrogen atom may play an important role in a ligand's ability to display differential binding affinity for 5-HT_2 versus 5-HT_{1C} receptors. Previously, only five compounds reported in multiple publications had been found to possess a greater than 10-fold selectivity for 5-HT_2 sites. By comparing the two-dimensional structures of these five compounds in the region hypothesized to be involved in receptor binding (i.e. the portion of the ligand containing the aromatic and nitrogen groups), we identified a carbonyl oxygen located between the aromatic and nitrogen groups which we speculated may play a role in receptor selectivity. It is of interest that these five compounds represent three distinct structural classes: butyrophenones (spiperone, haloperidol), benzoylpiperidinyl compounds (ketanserin, pirenperone), and a cinnamanilide (cinanserin). We therefore chose to test six additional compounds, three from the butyrophenone class (cloroperone, droperidol, pipamperone), and three from a distinct class of compounds (MDL 11939, MDL 26508, MDL 28133). All of these compounds contain the necessary carbonyl group, except for MDL 11939 and MDL 26508 which have a reactive oxygen within a carboxyl group in place of the carbonyl group.

All six of these compounds displayed greater than 20-fold selectivity for the 5-HT_2 versus 5-HT_{1C} receptor. Considering the large number of compounds which have been tested at these receptors, the ability to identify six selective compounds based on a single structural feature and thereby doubling the number of known selective compounds is a significant test of the feature's contribution to the ligands' selective role. In observing the receptor selectivity ratios in Table 2, it is apparent that the presence of the carbonyl or carboxyl group is not the only determinant of receptor selectivity. In fact, comparison of ratios between the six butyrophenone agents shows that structural differences beyond the key nitrogen also play a role in receptor selectivity since the butyrophenones are structurally identical below the key nitrogen.

Leysen (1990) recently published seven novel compounds found to be selective for 5-HT_2 versus 5-HT_{1C} receptors. Interestingly, five of the compounds (cisapride, altanserin, setoperone, oxatomide, pipamperone) contain a carbonyl group located between a phenyl ring and nitrogen atom. The two remaining compounds, risperidone and fluphenazine, do not contain a reactive oxygen in either a carbonyl or carboxyl group. As stated earlier, this reactive oxygen is not the only determinant of 5-HT_2 receptor selectivity, therefore it is not surprising that a 5-HT_2 selective agent may not contain this structural feature. However, nonselective compounds would not be expected to contain a carbonyl/carboxyl group interposed between the receptor-binding phenyl ring and nitrogen atom.

Since the reactive carbonyl or carboxyl group is located in the receptor-binding portion of the ligand, further speculation regarding receptor-ligand interactions can be made. The agents that we tested without a carbonyl or carboxyl group in the specified location all displayed high affinity for both receptors. Therefore, the presence of the reactive oxygen appears to be a hindrance to 5-HT_{1C} binding, perhaps via ionic interactions with unique 5-HT_{1C} receptor amino acids, thereby interfering with the normal receptor "docking" of the ligand's aromatic ring and nitrogen atom. This would result in relative selectivity of the reactive oxygen-containing drugs for 5-HT_2 receptors.

We chose to study the 5-HT_2 and 5-HT_{1C} receptors because of their similar amino acid sequences (80% homology in the transmembrane segments). Since other studies have shown that these transmembrane regions and their configurational arrangement are important in receptor-binding interaction, we compared the amino acid side chains within these segments for differences that would allow ionic interactions to occur between the reactive oxygen in the ligand and an amino acid within the 5-HT_{1C} receptor which is not present in the equivalent position in the 5-HT_2 receptor. The high degree of homology between these two receptors allows this comparison to be relatively straightforward.

Comparison of the cloned and sequenced rat 5-HT_2 receptor with the rat 5-HT_{1C} receptor (Pritchett et al. 1988) reveals that the nonpolar amino acid alanine (Ala 324), located in the 5-HT_2 sixth transmembrane segment toward the extracellular side, is replaced by the carboxyl side-chained serine in the 5-HT_{1C} receptor. Likewise, alanine (Ala 338) in the seventh transmembrane segment at the extracellular border of the 5-HT_2 receptor is replaced by the alkylammonium side-chained lysine in the 5-HT_{1C} receptor. All other amino acid differences between the 5-HT_2 and 5-HT_{1C} transmembrane segments do not involve changes in polarity of the amino acid side chain which would confer hydrogen bonding or other ionic interaction capabilities selectively for the 5-HT_{1C} receptor.

Although the 5-HT receptor subtypes analyzed in the present study are from different species (cow and pig),

5-HT receptor pharmacologic profiles between species reveals a high degree of similarity. Only select ergots, such as mesulergine and methysergide, differentiate between pig, rat and human 5-HT_2 receptors to a significant degree, while cinanserin modestly differentiates (Pazos et al. 1984b). However, we cannot exclude the possibility that species differences in receptor amino acids may play a role in ligand selectivity reported in the present study.

The substituted polar serine and lysine side chains within the sixth and seventh transmembrane segments of the 5-HT_{1C} receptor may interact with the carbonyl or carboxyl group of the 5-HT_2 selective ligands, thus, disrupting optimal docking of the aromatic ring and nitrogen atom, resulting in relative 5-HT_2 receptor selectivity. In support of our conclusion, Kobilka et al. (1988) have shown, using chimeric adrenergic receptors, that the transmembrane segments six and seven are the major determinants of antagonist ligand binding specificity. Additional studies involving single amino acid deletions from these receptors may further delineate the regions of the receptor specifically involved in receptor-ligand interactions.

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