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Pharmacological analysis of the novel, rapid and potent inactivation of the human 5-HT_7 receptor by risperidone, 9-OH-risperidone and other "inactivating antagonists"

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Running title: 5-HT_7 receptor inactivating antagonists

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Abstract:

In a previous publication, using h5-HT_7 receptor-expressing HEK293 cells, we reported the rapid, potent inactivation of the h5-HT_7 receptor stimulation of cAMP production by three antagonists: risperidone, 9-OH-risperidone, and methiothepin (Smith et al., 2006). In order to better understand the drug-receptor interaction producing the inactivation we a) expanded the list of inactivating drugs; b) determined the inactivating potencies and efficacies by performing concentration-response experiments and c) determined the potencies and efficacies of the inactivators as irreversible binding site inhibitors. Three new drugs were found to fully inactivate the h5-HT_7 receptor: lisuride, bromocryptine, and metergoline. As inactivators these drugs diplayed potencies of 1, 80, and 321nM, respectively. Pre-treatment of 5-HT_7-expressing HEK cells with increasing concentrations of the inactivating drugs risperidone, 9-OH-risperidone, methiothepin, lisuride, bromocryptine, and metergoline potently inhibited radiolabeling of the h5-HT_7 receptor, with IC_{50} values of 9, 5.5, 152, 3, 73, and 10nM, respectively. Surprisingly, maximal concentrations of risperidone and 9-OH-risperidone inhibited only 50% of the radiolabeling of h5-HT_7 receptors. These results indicate that risperidone and 9-OH risperidone may be producing 5-HT_7 receptor inactivation by different mechanisms than lisuride, bromocryptine, metergoline, and methiothepin. These results are not interpretable using the conventional model of GPCR function. The complex appears capable of assuming a stable inactive conformation due to the interaction of certain antagonists. The rapid, potent inactivation of the receptor-G-protein complex by antagonists implies a constitutive, pre-existing complex between the h5-HT_7 receptor and a G-protein.

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Introduction:

The 5-HT_7 receptor is one of 13 5-HT receptors expressed in mammalian tissues (Teitler and Herrick-Davis, 1994;Gerhardt and van Heerikhuizen, 1997;Hoyer and Martin, 1997;Hoyer *et al.*, 2002;Kroeze *et al.*, 2002;Raymond *et al.*, 2001). It was discovered through homology cloning and is expressed in various areas of the human brain and in peripheral tissues, including important blood vessels in the cerebral vasculature (Bard *et al.*, 1993;Lovenberg *et al.*, 1993;Shen *et al.*, 1993;Teitler and Herrick-Davis, 1994;Hedlund and Sutcliffe, 2004). 5-HT_7 receptor antagonists are being developed for possible use in various clinical conditions including migraine (Terron, 1997), sleep (Lovenberg *et al.*, 1993), psychosis (Bard *et al.*, 1993;Lovenberg *et al.*, 1993;Shen *et al.*, 1993), and depression (Bard *et al.*, 1993;Lovenberg *et al.*, 1993;Shen *et al.*, 1993;Hedlund and Sutcliffe, 2004).

Risperidone is a highly prescribed atypical antipsychotic drug (Love and Nelson, 2000;Bhana and Spencer, 2000;Gilbody *et al.*, 2000;Green, 2000;Schneider *et al.*, 2006). It is one of a group of drugs believed to initiate their effects through interactions with the D_2 dopamine and 5-HT_{2A} serotonin receptors (Roth *et al.*, 1994;Meltzer *et al.*, 1989). These interactions have been shown to be classical competitive antagonist interactions (Smith *et al.*, 2006;Roth *et al.*, 1994). Recently we reported that risperidone, 9-OH-risperidone, the active metabolite of risperidone (Borison *et al.*, 1994;Ereshefsky and Lacombe, 1993;Spina *et al.*, 2001), and methiothepin, a classical, non-selective 5-HT receptor antagonist, produce a unique effect on cells expressing the h5-HT_7 receptor (Smith *et al.*, 2006). Pre-treatment with these three drugs at low concentrations for a short time (30 min) produces a long-lasting inactivation of the h5-HT_7 receptor. ^{3}H-risperidone binding studies indicated that this effect is due to an irreversible complex formed between risperidone and the h5-HT_7 receptor. It is presumed that a similar mechanism is responsible for the effects of 9-OH-risperidone and methiothepin, although this can only be indirectly demonstrated as radiolabeled forms of these latter two drugs are not available. Interestingly, exposing membrane

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homogenates prepared from h5-HT_7-expressing cells to risperidone or 9-OH-risperidone did not produce an irreversible occlusion of the binding site (Smith *et al.*, 2006). These results indicated the receptor must be in the cellular environment in order to irreversibly bind these drugs.

Nine drugs studied at the same time as risperidone, 9-OH-risperidone, and methiothepin did not display the inactivating properties (Smith *et al.*, 2006). In order to more fully examine the pharmacological properties of drugs that promote the irreversible interaction with the h5-HT_7 receptor, we attempted to expand the list of inactivating drug. 17 drugs not previously studied were screened for inactivating properties. These drugs were selected based on having high to moderate affinities for the 5-HT_7 receptor, assuring a drug-receptor interaction at nM-µM concentrations (Monsma, Jr. *et al.*, 1993;Bard *et al.*, 1993;Ruat *et al.*, 1993;Roth *et al.*, 1994;Shen *et al.*, 1993;Lovenberg *et al.*, 1993). Three new inactivating drugs were discovered. The ability of the inactivating drugs to inhibit radioligand binding as well as 5-HT-stimulated activity was monitored (after drug removal) in order to determine the relationship between receptor occupancy and inactivating potency. Pre-treatment with all six inactivators eliminated all h5-HT_7 receptor-mediated activity. Pre-treatment with bromocryptine, metergoline and methiothepin eliminated all radiolabeling of h5-HT_7 receptors; lisuride eliminated ~80% of the radiolabeled receptors. However, risperidone and 9-OH-risperidone only maximally inhibited 50% of the radiolabeled h5-HT_7 receptors. Taken together these results indicate the possibility of two different mechanisms of h5-HT_7 receptor inactivation amongst the six inactivating drugs.

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Materials and Methods:

Homogenate radioligand binding.: Radioligand binding studies in membrane homogenates were performed as described previously with modifications (Smith *et al.*, 2006;Purohit *et al.*, 2005). HEK cells stably expressing h5-HT_7 receptors (100 mm dish; ~100% confluent) were scraped and collected in 50 mM Tris-HCl, 0.5 mM EDTA, and 10 mM MgSO4, pH 7.7 (at 23°C); centrifuged at 14,000×*g* for 30 min; homogenized using a Polytron homogenizer (Kinematica, Basel, Switzerland); and centrifuged again at 14,000×*g* for 30 min. The membranes were resuspended in HEPES buffer (20 mM HEPES , 2.5 mM MgSO4, 134mM NaCl, pH 7.5 , 23°C) and 0.1% ascorbic acid. Assays were performed in triplicate 1.0-ml volumes containing 10 µg of membrane protein (which was added last). Assays containing 2 nM ^{3}H-5-HT (24 Ci/mmol; PerkinElmer Life and Analytical Sciences, Boston, MA) were performed in the absence and presence of 10 µM clozapine to detect the level of available h5-HT_7 receptors. Specific binding was generally 85–95% of total binding. Tubes were incubated for 30 min at 37°C, filtered, and counted using liquid scinitillation. Experimental results were analyzed using Prism 5.0 (GraphPad Software, San Diego, CA). Protein content of the samples was determined with the use of a BCA protein assay kit (Pierce Chemical, Rockford, IL).

Whole cell radioligand binding: Whole cell radioligand binding studies were performed as described previously with modifications (Smith *et al.*, 2006;Kong *et al.*, 2006). Cells were lifted using 1 ml/dish diluted trypsin-EDTA, followed by the addition of 5 ml/dish of HEPES buffer (see above). Cells were gently centrifuged for 3 min at 330×*g*, supernatant was aspirated and cells resuspended in HEPES buffer. Cells were pre-treated with drug, incubated 30 min at 37°C, washed 3×10min with HEPES buffer, resuspended in HEPES buffer and added to the assay tubes. Assay tubes contained 2nM ^{3}H-5-HT for competition studies or varying concentrations (0.5-15nM) for saturation analyses. 10µM clozapine was

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used to define non-specific binding. Assay tubes were incubated for 30 min at 37°C, filtered, and counted using liquid scintillation.

cAMP Assay: Total cAMP accumulation was measured using the LANCE cAMP Detection kit (Perkin-Elmer). Cells were lifted using 1ml/dish Versene, followed by the addition of 11ml/dish Hanks Buffered Saline Solution. Cells were centrifuged for 3 min at 330×*g*, supernatant was aspirated and the cells were resuspended in HEPES buffer. Cells were pre-treated with drug, incubated 30 min at 37°C, washed 3×10 min in HEPES buffer. After the third wash, cells were resuspended in stimulation buffer (prepared according to the Perkin-Elmer LANCE cAMP instruction manual). Cells were counted with a hemacytometer and added to 96-well white opaque plates. The pre-treated cells were then exposed to 10μM 5-HT for 30 min at 23°C. Control experiments demonstrated that this procedure produced no effect on the cells' responsiveness to 5-HT (see Results). Time-resolved fluorescence resonance energy transfer was detected by the Victor3 1420 plate-reader (Perkin-Elmer).

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Results:

Figure 1 displays the results of screening 20 drugs for inactivating properties. These drugs were selected based on preliminary radioligand binding studies indicating they had high to moderate affinities for the h5-HT_7 receptor. The h5-HT_7 receptor expressing HEK cells were first exposed to 1μM drug for 30 min, followed by three washouts. The cells were then exposed to 10μM 5-HT for 30min. Inactivation was defined as the inability of the cells to produce cAMP in response to the 10 μM 5-HT stimulation. Six of the drugs tested displayed this property: risperidone, 9-OH-risperidone, and methiothepin (previously characterized as inactivators (Smith et al., 2006)) and lisuride, bromocryptine, and metergoline, which were not previously characterized. The six drugs tested that had inactivating ability exhibited h5-HT_7 receptor affinities that ranged from 0.4nM-143nM. Lisuride, (0.4nM), risperidone (2nM), methiothepin (3nM), 9-OH-risperidone (10nM), metergoline (16nM), and bromocryptine (143nM) displayed inactivating properties (figures 1&2). Other drugs tested that did not display inactivating properties are listed with their h5-HT_7 receptor affinities (nM) :amoxapine (69); amitryptiline (96); cyproheptadine (24); loxapine (258); mianserin (64); ritanserin (468); the selective 5-HT_7 receptor antagonist SB269970 (2); tenilapine (153); TFMPP (1624); trifluperazine (497); the high affinity 5-HT_2 receptor antagonist ICI169369 (393); clozapine (30); LSD (3), and methysergide (32). Of the six inactivators only metergoline produced less than a complete inactivation at 1μM, indicating it is either less potent than the other five inactivators, or is producing its inactivation through a distinct and less efficacious mechanism.

In order to obtain more information on this novel inactivation property of the six drugs, concentration-response curves for h5-HT_7 inactivation were produced (figure 2; table 1). The h5-HT_7 receptor expressing HEK cells were first exposed to increasing concentrations of drug for 30 min, followed by three washouts. The cells were then exposed to 10μM 5-HT for 30min. Risperidone, 9-OH-risperidone, lisuride, and methiothepin displayed high potencies for producing the inactivation effect, with IC_{50} values

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of 1.3nM, 3.9nM, 1.0nM, and 3.0nM, respectively. Bromocryptine and metergoline displayed lower potencies with IC_{50} values of 80nM and 321nM, respectively. Five of the six inactivating drugs were able to fully inactivate the receptor at 10uM concentrations. Metergoline produced a maximal inhibition of activity of ~75%. The potencies of the inactivators closely corresponded with their affinities for the h5-HT_7 receptor with the exception of metergoline. Metergoline is a high affinity ligand for the h5-HT_7 receptor (Ki=16nM). Thus it appears that the potency of inactivation involves more than occupancy of the receptor, possibly involving differing inactivation kinetics for different inactivators (see below).

In order to explore the relationship between receptor occupancy and receptor inactivating activity for the six inactivators, we incubated increasing concentrations of the six inactivators with the h5-HT_7 receptor-expressing cells, thoroughly washed the cells, and then measured receptor occupancy using ^{3}H-5-HT to label the receptors (figure 3). While bromocryptine, metergoline, and methiothepin produced a potent and complete inhibition of binding, risperidone and 9-OH-risperidone produced surprising concentration-response curves. Maximal concentrations of risperidone and 9-OH-risperidone inhibited 50% of the receptor binding, despite the previous observation that these drugs potently and completely inactivate the h5-HT_7 receptors (figure 2). Lisuride pre-treatment also produced less than a complete loss of binding sites, inhibiting 82±4 % of the binding, which was a significantly different effect from any of the other inactivators (figure 3).

In order to explore the properties of the ^{3}H-5-HT binding remaining after pre-treatment with risperidone and 9-OH-risperidone (figure 3), ^{3}H-5-HT competition curves (figure 4) and saturation analyses (figure 5), were performed after drug pre-treatment and three washes. As shown in figure 4 the pharmacological properties of the remaining specific ^{3}H-5-HT binding is that of the h5-HT_7 receptor, displaying high affinity for the selective 5-HT_7 antagonist SB269970. Saturation analyses of high affinity ^{3}H-5-HT binding indicate that ~50% of the binding was removed, with no change in affinity for ^{3}H-5-HT (figure

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5). Thus pre-treatment with risperidone and 9-OH-risperidone can completely inactivate the h5-HT_7 receptor-mediated response but can only irreversibly block 50% of the h5-HT_7 receptors.

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Discussion:

The effects of risperidone, 9-OH-risperidone, lisuride, bromocryptine, methiothepin and metergoline on the h5-HT_7 receptors expressed in HEK-298 cells display several novel characteristics. Most dramatic is the rapid, potent, and complete inactivation of the receptor after exposure to these drugs (figures 1&2). While a majority of the 20 drugs tested to date in this series of studies display classical competitive antagonism, these six drugs clearly produce a drug-receptor interaction that results in an inactivated receptor. In earlier studies with ^{3}H-risperidone, a pseudo-irreversible interaction with the h5-HT_7 receptor was directly demonstrated (Smith *et al.*, 2006). This interaction apparently explains the inactivation effect. Presumably the five other inactivating drugs produce a similar pseudo-irreversible effect. Due to the lack of radiolabeled forms of these drugs, this effect cannot be directly demonstrated. The structural features of the six inactivating drugs that allow the production of the pseudo-irreversible complex with the h5-HT_7 receptor are not immediately obvious as the six drugs represent diverse chemical families. Risperidone and 9-OH-risperidone (benzisoxazole derivatives) are similar in structure and both produce the inactivating effect. Lisuride, bromocryptine, and metergoline are ergoline derivatives. However LSD, another ergoline derivative, does not produce the inactivating effect (legend, figure one). Methiothepin is a methylpiperazine with no obvious similarities to risperidone or the ergolines. Possibly computer modeling of the structures might reveal a similar structural motif not obvious in a two-dimensional figure. It was anticipated that performing radioligand binding studies after exposing the cells to the inactivating ligands would produce potent inhibition of the radioligand binding signal, reflective of the potent inactivating properties of these drugs. However, as shown in figures 2&3, the inactivating drugs fell into two groups. Methiothepin, metergoline, and bromocryptine, produced the anticipated potent and complete inhibition of radioligand binding predicted for drugs pseudo-irreversibly complexed to the h5-HT_7 receptor. Lisuride produced a potent and nearly complete inhibition (figure 3). However risperidone and

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9-OH-risperidone produced a puzzling effect. Both drugs potently inhibited 50% of the receptor binding. Thus 50% of the h5-HT_7 receptors appear to be risperidone and 9-OH-risperidone resistant (figures 3&5). This resistant binding appears to be composed of intact h5-HT_7 receptors, based on the affinity SB-266970 (figure 4).

The results in figures 2&3 indicate there are at least two slightly different mechanisms of inactivation occurring. While the six inactivators can inhibit all the functional activity of h5-HT_7 receptors, as judged by 5-HT-stimulated cAMP production, risperidone and 9-OH-risperidone produce only a 50% loss of ^{3}H-5-HT binding sites. The other four inactivators are efficacious inhibitors of ^{3}H-5-HT specific binding to h5-HT_7 receptors (figure 3). These results imply that the six inactivators share the ability to stabilize a conformation of the receptor that inactivates Gs. This inactivation appears to be very stable, presumably due to the pseudo-irreversible interaction of the inactivators with the h5-HT_7 receptor revealed in a previous publication (Smith *et al.*, 2006). The rationale for the loss of 50% of the binding sites, rather than all the binding sites, due to the pre-treatment with risperidone or 9-OH-risperidone is under investigation. These results imply a possible receptor-receptor interaction modified by risperidone and 9-OH-risperidone. Given the 50% effect observed, the possibility of the involvement of dimerization seems likely. One possibility is that the binding of risperidone or 9-OH-risperidone to one protomer of a dimer pair produces an allosteric effect on the other protomer. The second protomer would presumably lose its ability to irreversibly bind risperidone or 9-OH-risperidone, thus leaving 50% of the sites available for radiolabeling. This possibility is currently under investigation.

Another surprising result is the relatively low potency of metergoline relative to the other inactivators. Metergoline is a high affinity ligand (Ki=16nM; table one), but displayed a relatively low potency inactivation (IC_{50}=112nM; figure two). Bromocryptine also displayed a lower potency as an inactivator but it also displays a lower affinity (Ki=143nM) than the other inactivators. It may be that each

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inactivating drug has a different time course for producing the pseudo-irreversible complex and that increasing the incubation time for metergoline will produce a higher potency interaction.]

The h5-HT_7 receptor has displayed unusual properties since its discovery through homology cloning(Bard *et al.*, 1993;Lovenberg *et al.*, 1993;Shen *et al.*, 1993). The original characterization revealed that this receptor displays high affinities for agonists, whether the radiolabel is an agonist or antagonist ligand. This is contrary to most group I GPCR, which display lower affinities for agonists when antagonist radioligands are used(Lyon *et al.*, 1987). Several studies have indicated that a 5-HT_7-receptor/G-protein complex may exist in the absence of an agonist (Bruheim *et al.*, 2003;Kvachnina *et al.*, 2005;Krobert *et al.*, 2001). Of special interest is a report that methiothepin produces an irreversible inhibition of the h5-HT_7 receptor (Krobert *et al.*, 2006). However the authors of that paper attribute the methiothepin effect to high lipophilicity producing a slow dissociation. The washout studies performed in the studies reported herein remove >90% of the drugs (data not shown). Thus residual drug is not a likely mechanism for the inability of 10μM 5-HT to stimulate the h5-HT_7 receptor subsequent to exposure to methiothepin or the other inactivating drugs. It is likely that the "persistent" loss of activity and binding sites observed after methiothepin pre-treatment (Krobert *et al.*, 2006) was due to the unusual interaction of methiothepin with the h5-HT_7 receptor as described herein.

In examining the literature for GPCRs that have been reported to produce some of the novel effects reported herein, a member of the neuropeptide Y receptor family appears to be of interest. The neuropeptide Y receptor family is a member of the type 1 GPCR family and is composed of four receptors, Y1, Y2, Y4, and Y5 (Ouedraogo *et al.*, 2008). Two observations reported for the Y2 receptor are intriguing. Most notably, neuropeptide Y and a small molecular antagonist BIIE0246 react irreversibly with the Y2 receptor in radioligand binding studies (Dautzenberg and Neysari, 2005). BIIE0246 irreversibly inactivates the Y2 receptor, in a manner very similar to the inactivators of the h5-HT_7

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receptor reported herein (Dautzenberg and Neysari, 2005). Another similarity is that, unlike the other members of the neuropeptide Y receptor family, the Y2 receptor does not internalize upon agonist stimulation (Dautzenberg and Neysari, 2005). The h5-HT_7 receptor also does not internalize in the presence of 10μM 5-HT as determined using GFP-tagged h5-HT_7 receptors to monitor receptor trafficking with confocal microscopy (Smith *et al.*, 2006). It may very well be that there are other members of the type 1 GPCR family that possess the unusual properties demonstrated by the h5-HT_7 receptor. However revealing these properties requires the appropriate drug(s) and the appropriate experiments. It is possible that GPCRs from diverse sub-groupings may possess structural components that allow the pseudo-irreversible interaction with endogenous ligands and/or antagonists., Drugs that interact irreversibly with these receptors may produce therapeutic and/or side-effects not seen with competitive antagonists.

Results of a study examining 5-HT_7 receptor pharmacology produced observations consistent with the results reported herein (Terron, 1997). Canine cerebral artery vasodilation was demonstrated to be mediated by the 5-HT_7 receptor through the use of antagonists to reverse 5-HT-mediated vasodilation. Three drugs were found to produce non-competitive antagonism: risperidone, methiothepin, and lisuride (Terron, 1997). Consistent with this report, we observe irreversible effects with these drugs. Interestingly, the 1997 report found metergoline to be a competitive antagonist (Terron, 1997). While this is apparently inconsistent with our results in which metergoline was found to produce an irreversible effect, a closer examination of our data indicates this is not actually inconsistent. Relative to the other irreversible antagonists, metergoline is a far lower potency irreversible antagonist than expected from its affinity (figure 2). The low dosages used in the 1997 study would not be expected to produce the irreversible effect we observed at relatively high concentrations of metergoline.

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In summary, the results presented indicate drug-receptor interactions previously unobserved in the monoamine GPCR family. The pseudo-irreversible interaction of a significant population of drugs (~29% of the drugs tested to date) with the receptor, producing a complete inactivation, has not been reported for any GPCR. These results may indicate there is a novel class of drugs, "GPCR inactivators", sharing the properties of the six drugs described herein. The ability of risperidone and 9-OH-risperidone to completely inhibit functional activity of the h5-HT_7 receptor, while only occluding 50% of the binding sites is also a novel observation. This may involve functional receptor-receptor interactions not previously studied.

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Footnotes:

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Figure Legends:

Figure 1: Effect of drug pre-treatment on h5-HT_7-receptor stimulated cAMP production. HEK-293 cells stably expressing h5-HT_7 receptors were suspended in isotonic buffer and exposed to 1μM concentrations of drugs for 30 min. Cells were gently pelleted, buffer was replaced and the cells exposed to non-drug-containing buffer. This drug washout procedure was repeated three times. Cells were resuspended and assayed for response to 10μM 5-HT using the LANCE cAMP Detection kit (Perkin-Elmer) and time-resolved fluorescence resonance energy transfer. The results are the means ± SEM of three independent experiments performed in triplicate. The following drugs (1μM) displayed no effect on h5-HT_7-receptor mediated stimulation of cAMP after washout: ICI169369, tenilapine, cyproheptadine, SB269970, TFMPP, trifluperazine, methysergide, ritanserin, loxapine, amoxapine, amitriptyline, and LSD.

$^{*}p<0.003$ vs. control

Figure 2: Concentration-response curves for inactivation of h5-HT_7 receptor stimulated cAMP production. HEK-293 cells expressing h5-HT_7 receptors were suspended in isotonic buffer and exposed to buffer (control) or varying concentrations of the six drugs displaying inactivating properties (see figure 1). After the drug washout procedure (see Materials and Methods) the cells were exposed to 10μM 5-HT for 30 min. cAMP levels were determined using the LANCE cAMP Detection kit (Perkin-Elmer). The results are the means ± SEM of three independent experiments performed in triplicate.

Figure 3: Concentration-response curves for loss of specific ^{3}H-5-HT binding to h5-HT_7 receptor after drug removal. HEK-293 cells expressing h5-HT_7 receptors were suspended in isotonic buffer and exposed to buffer (control) or varying concentrations of the six drugs displaying inactivating properties (see figure 1). After the drug washout procedure (see Materials and Methods) the cells were incubated with 2nM ^{3}H-

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5-HT in the presence and absence of 10μM clozapine. The results are the means ± SEM of three independent experiments performed in triplicate.

Figure 4: Affinities of SB-269970 for ^{3}H-5-HT specific binding after pre-treatment with no drug (control), 1μM risperidone or 1μM 9-OH-risperidone. Consistent with figures 3&5, 50% of the h5-HT_7 receptors are risperidone and 9-OH-risperidone resistant. The high affinities displayed by SB-269970 indicate the risperidone and 9-OH-risperidone-resistant radioligand binding signal displays the pharmacological properties of the h5-HT_7 receptor. The results are the means ±SEM of three independent experiments.

Figure 5: Saturation analyses of specific ^{3}H-5-HT binding to HEK-293 cells expressing the h5-HT_7 receptor, after pre-treatment with no drug (control), 1μM risperidone, or 1μM 9-OH-risperidone. After drug pre-treatment and washout procedure cells were incubated with increasing concentrations of ^{3}H-5-HT in the absence and presence of 10μM clozapine. The results are the means ± SEM of three independent experiments performed in triplicate.

*$p<0.001$ vs. control Bmax values.

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Table one: comparison of affinities of the six inactivating drugs for the h5-HT_7 receptor in radioligand binding assays with their potencies as insurmountable inhibitors of h5-HT_7 receptor binding or inhibitors of h5-HT_7 receptor activity. IC_{50} values are reported as the receptors are tested subsequent to the removal of the inactivating drug. Results are the means ± SEM of three independent experiments performed in triplicate.

drug	Ki (nM)[a]	IC_{50} (nM)[b]	IC_{50}(nM)[c]
risperidone	1.8±0.3	9±4	1.3±0.5
9-OH-risperidone	10±1.7	5.5±3.6	3.9±2.1
bromocryptine	143±56	152±52	80±22
lisuride	0.4±0.2	3.0±1.1	1.0±0.3
metergoline	16±2	73±22	321±130
methiothepin	3.0±0.5	9.8±1.3	3.0±1.7

[a] h5-HT_7 affinity (membrane homogenate radioligand binding)

[b] insurmountable inhibition of h5-HT_7 whole cell binding

[c] insurmountable inhibition of h5-HT_7-receptor-mediated cAMP accumulation

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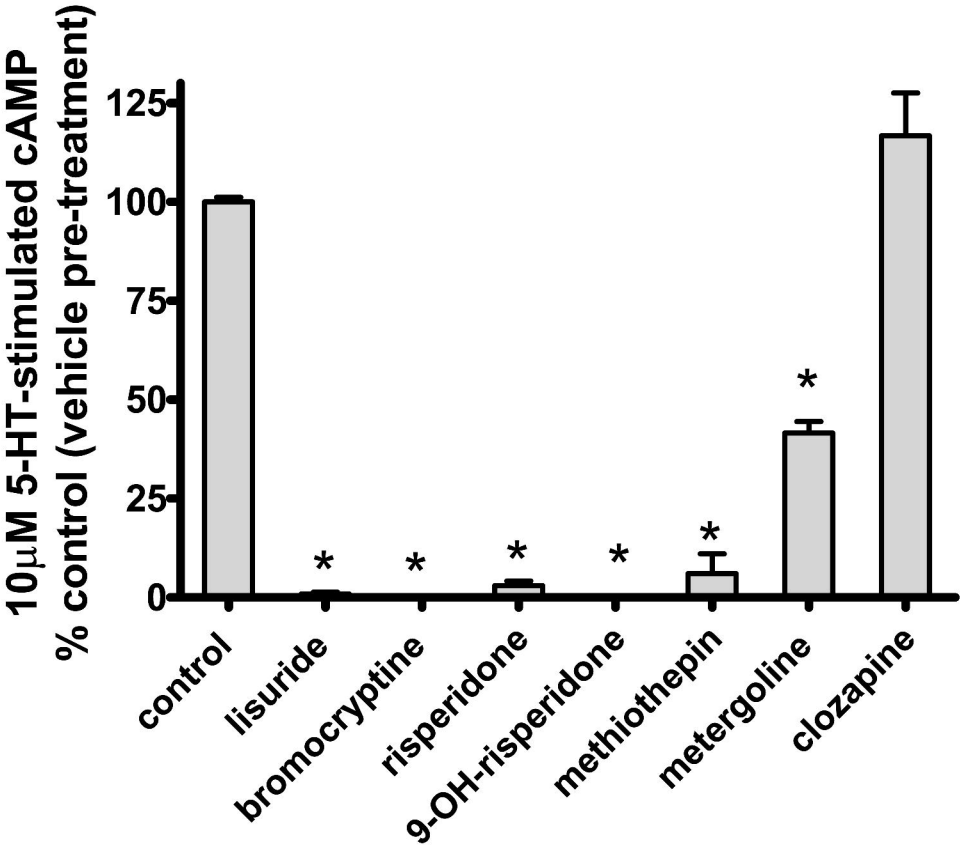

10μM 5-HT-stimulated cAMP
% control (vehicle pre-treatment)
125
100
75
50
25
0
*
*
*
*
*
*
control
lisuride
bromocryptine
risperidone
9-OH-risperidone
methiothepin
metergoline
clozapine

Figure one

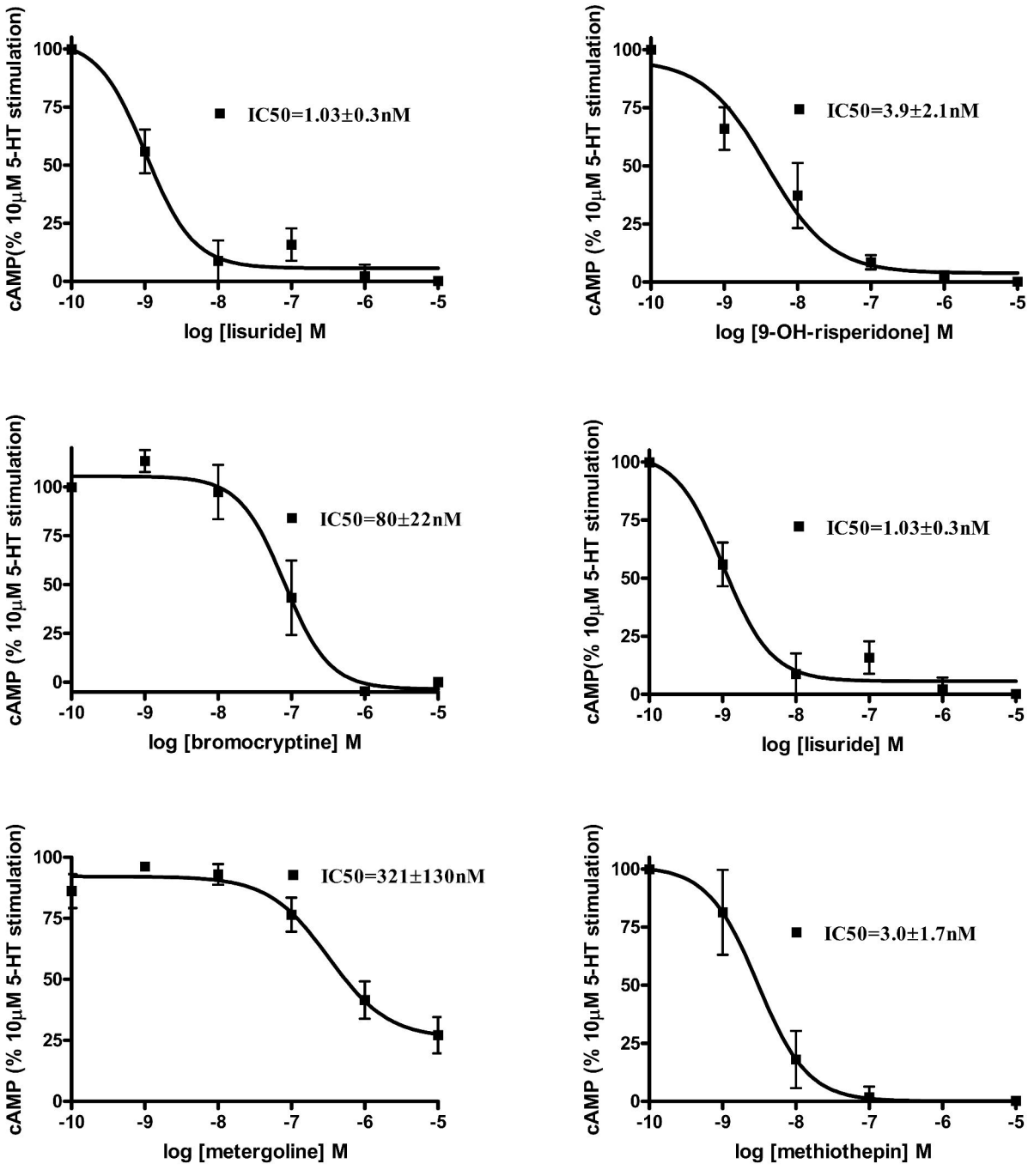
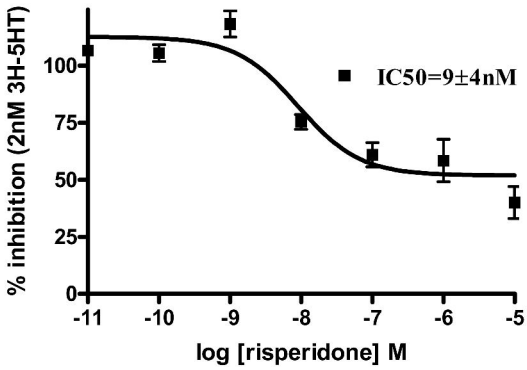
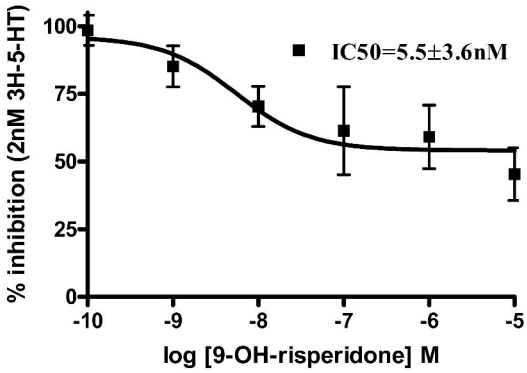
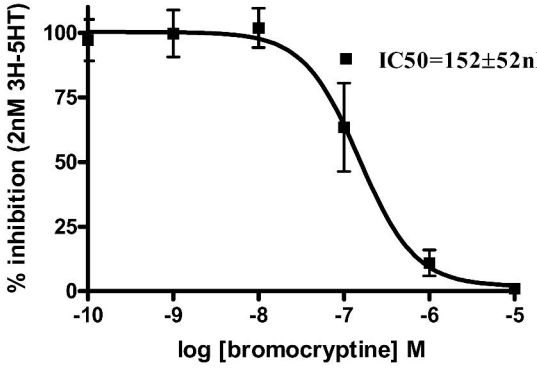
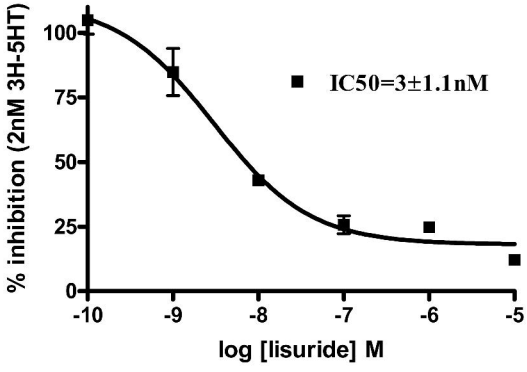

cAMP(% 10μM 5-HT stimulation)
IC50=1.03±0.3nM
log [lisuride] M
cAMP (% 10μM 5-HT stimulation)
IC50=3.9±2.1nM
log [9-OH-risperidone] M
cAMP (% 10μM 5-HT stimulation)
IC50=80±22nM
log [bromocryptine] M
cAMP(% 10μM 5-HT stimulation)
IC50=1.03±0.3nM
log [lisuride] M
cAMP (% 10μM 5-HT stimulation)
IC50=321±130nM
log [metergoline] M
cAMP (% 10μM 5-HT stimulation)
IC50=3.0±1.7nM
log [methiothepin] M
100
75
50
25
0
-10
-9
-8
-7
-6
-5

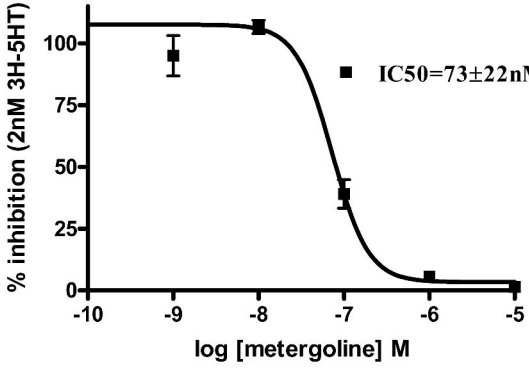
Figure two











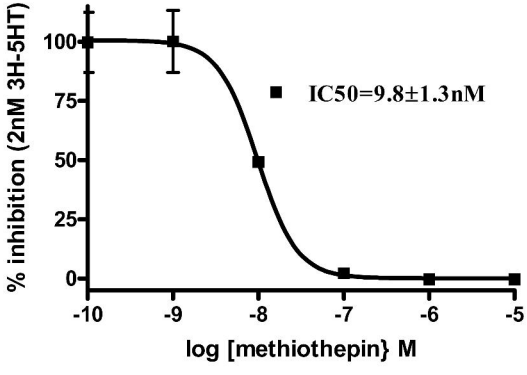


Figure three

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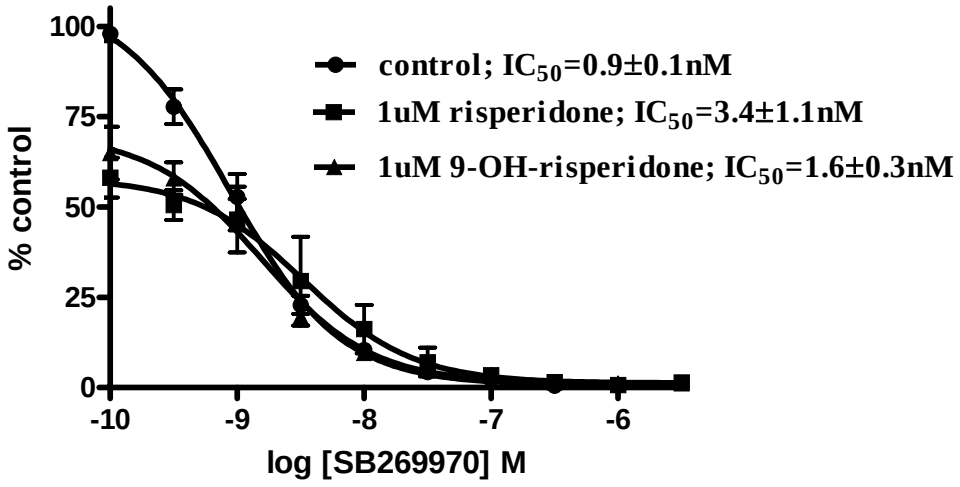

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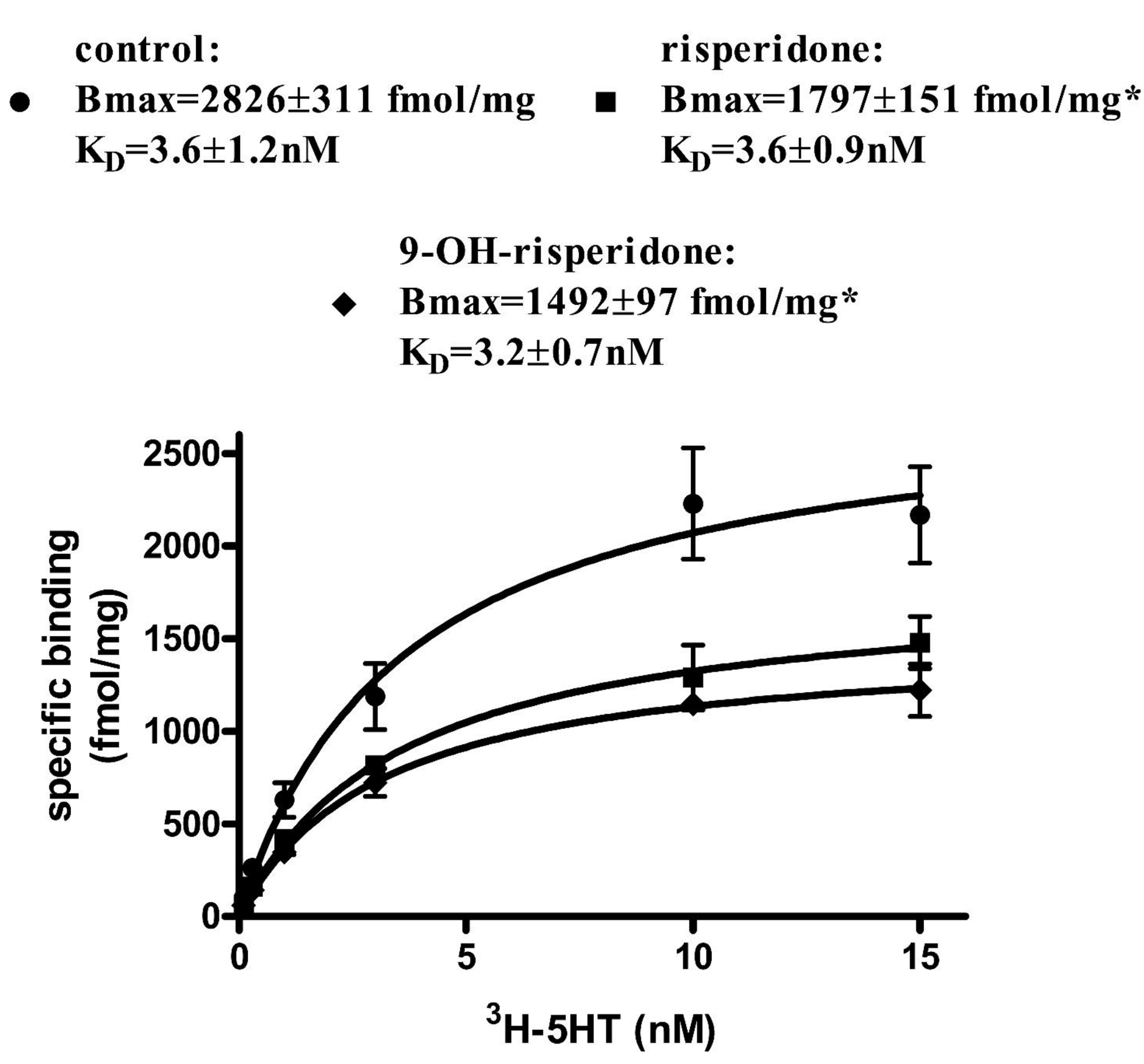
control:
Bmax=2826±311 fmol/mg
K_D=3.6±1.2nM
risperidone:
Bmax=1797±151 fmol/mg*
K_D=3.6±0.9nM
9-OH-risperidone:
Bmax=1492±97 fmol/mg*
K_D=3.2±0.7nM
specific binding (fmol/mg)
2500
2000
1500
1000
500
0
0
5
10
15
^{3}H-5HT (nM)

Figure five