N-Substituted (2,3-Dihydro-1,4-benzodioxin-2-yl)methylamine Derivatives as D_2 Antagonists/5-HT_{1A} Partial Agonists with Potential as Atypical Antipsychotic Agents

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A series of *N*-substituted 1-(2,3-dihydro-1,4-benzodioxin-2-yl)methylamine derivatives with D_2 antagonist/5-HT_{1A} partial agonist activity has been prepared as potential atypical antipsychotic agents. Optimization of in vitro receptor binding activity and in vivo activity in rodent models of psychosis has led to compound **24**, which showed good affinities for human D_2, D_3, and 5-HT_{1A} receptors but significantly less affinity for human α_1 adrenoceptors and rat H_1 and muscarinic receptors. In rodents, **24** showed functional D_2-like antagonism and 5-HT_{1A} partial agonism. After oral dosing, **24** showed good activity in rodent antipsychotic tests and very little potential to cause extrapyramidal side effects (EPS), as measured by its ability to induce catalepsy in rats only at very high doses. In the light of this promising profile of activity, **24** has been selected for clinical investigation as a novel antipsychotic agent with a predicted low propensity to cause EPS.

Introduction

Schizophrenia is a relatively common disorder, affecting approximately 1% of the population, and one where established therapies leave much room for improvement. Progress is still sought in terms of efficacy, against both the positive symptoms of the disease, such as delusions and hallucinations, and the negative symptoms, such as social withdrawal and flattened affect, and also in terms of lower side effect potential. There is strong evidence[1] that classical antipsychotic agents, such as haloperidol (Chart 1), exert their beneficial effects through blockade of central dopamine D_2 receptors. In addition, the acute extrapyramidal side effects (EPS) induced by these agents, such as Parkinsonism, akathisia, and dystonia, are also thought to result from their high degree of D_2-receptor antagonism.

Clozapine is exceptional among established antipsychotic agents, as it combines effectiveness against both positive and negative symptoms of the disease with a very low propensity to induce EPS. It has significant affinity for a broad range of neurotransmitter receptors,[2] which seems to account for its clinical effectiveness at a relatively low occupancy of D_2 receptors (40−60%),[1] which in turn is probably responsible for the lack of EPS seen with the drug. Clozapine was withdrawn from the market over 20 years ago due to a relatively high incidence of potentially fatal agranulocytosis, but was reintroduced more recently, under carefully controlled conditions, for the treatment of patients who failed to respond to other drugs.

The search for alternatives to clozapine, which match its effectiveness but lack its serious side effects, has led to the structurally related compounds zotepine,[3] olanzapine,[4] and quetiapine,[5] which are all newly approved for the treatment of schizophrenia in major markets. An alternative approach to the discovery of atypical antipsychotics has been to target the other newly identified dopamine receptor subtypes, e.g., D_1/D_5, D_3, and D_4, which would not be expected to be associated with EPS.[1] However, although a number of selective, high-affinity compounds have been described, none of these has yet been reported to be clinically effective. More successful has been the combination of D_2-receptor antagonism with serotonergic activity. For example, in the recently introduced drugs risperidone[6] and sertindole,[7] 5-HT_{2A}-receptor antagonism is believed to play an important role in their activity.

Typical D_2-receptor antagonists, such as haloperidol and raclopride, induce catalepsy in rats, an activity which is predictive of the propensity of compounds to cause EPS when administered to man. Following the demonstration that the 5-HT_{1A}-receptor full agonist 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) reversed this catalepsy,[8] we sought to find molecules which combined antagonism at D_2-like receptors with agonism or partial agonism at 5-HT_{1A} receptors. We required these compounds to have much lower affinity

Chart 1

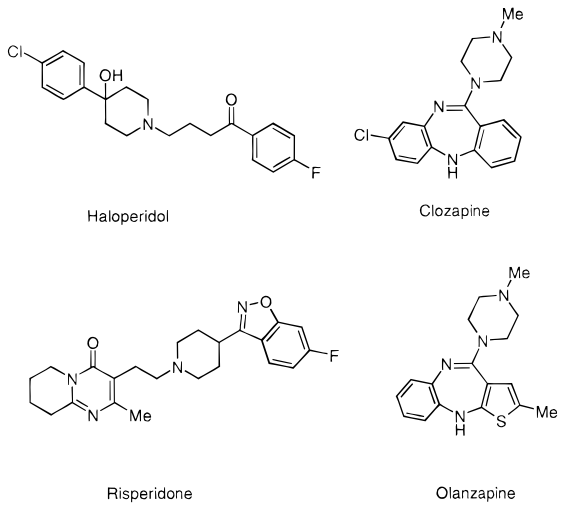

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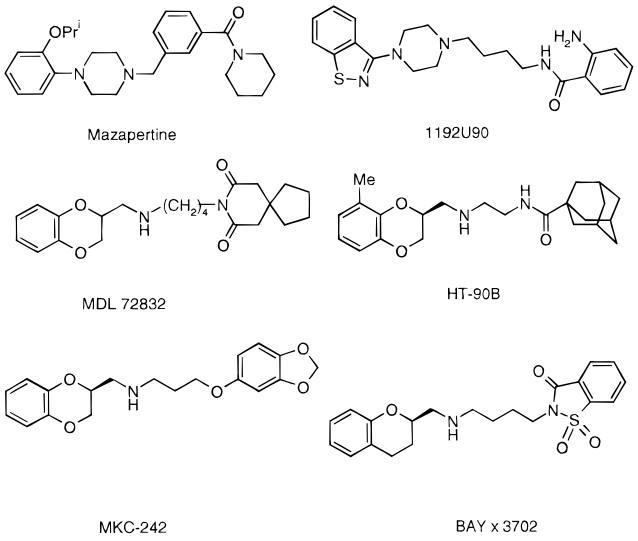
Chart 2



for receptors associated with side effects, such as α_1 adrenoceptors (sedation, postural hypotension), histamine H_1 (sedation), and muscarinic (dry mouth, blurred vision).

Other research groups have also targeted D_2-antagonist/5-HT_{1A}-agonist compounds as potential antipsychotics with low EPS. At least two such compounds, RWJ-37796 (mazapertine, Chart 2)[9] and 1192U90[10] have reached early clinical development. These two compounds are arylpiperazine derivatives which show high and approximately equal affinities for D_2, 5-HT_{1A}, and α_1 receptors. The clinical undesirability of high affinity for α_1 adrenoceptors was reinforced by reports that mazapertine[11,12] had encountered problems with postural hypotension in clinical trials.

The arylpiperazine moiety found in the compounds above would appear to be isosteric with the *N*-(2,3-dihydro-1,4-benzodioxin-2-yl)methylamine substructure, as evidenced by accounts of several derivatives of the latter type which also have activity at various dopaminergic, serotoninergic, and noradrenergic receptor subtypes. Often, compounds of this type show significant affinity for D_2, 5-HT_{1A}, and α_1 receptors, although the relative proportion varies with the particular structural class. For example, the spirocyclic imide derivatives such as MDL 72832 and its aza-analogues[13] show selectivity for the 5-HT_{1A} receptor, though with still significant affinities for D_2 and α_1 receptors. Comparison of the enantiomers of MDL 72832 with other standard 5-HT_{1A} ligands, using molecular modeling techniques,[14] allowed Hibert and co-workers to derive a pharmacophore for that receptor. The model correctly predicted that the (*S*)-enantiomer would have the higher affinity. The 8-methyl-2,3-dihydro-1,4-benzodioxin derivative (−)HT-90B is structurally related to the compound above and shares a similar profile, with some selectivity for 5-HT_{1A} but still significant affinities for D_2 and α_1 receptors.[15] MKC-242 is another benzodioxin derivative, and although it lacks a polar amide group in its side chain, its receptor binding profile remains similar.[16]

In contrast to these, a series of 7-hydroxy-2,3-dihydrobenzodioxin derivatives, together with their chroman and tetralin analogues, were found to show some preference for the D_2 receptor (high-affinity form) and to show agonist properties.[17] Again the (*S*)-(−)-forms showed the higher affinities.

The chroman derivative BAY x 3702 is, in general structural terms, similar to MDL 72832 and is also a potent 5-HT_{1A} ligand with some selectivity over α_1 and D_2.[18] A recent report describes aminotetralin derivatives, which may be regarded as hybrid structures between 8-OH-DPAT and the amides described above, as having more balanced affinities for 5-HT_{1A} and D_2 receptors.[19]

We report here a new series of *N*-substituted 1-(2,3-dihydro-1,4-benzodioxin-2-yl)methylamine derivatives, the best of which have good affinity and selectivity for the D_2 and 5-HT_{1A} receptors and show potential for atypical antipsychotic activity in animal models.

Chemistry

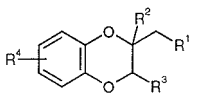
The majority of the target compounds described in this study (Table 1) were prepared either directly or indirectly by reaction of the (2,3-dihydro-1,4-benzodioxin-2-yl)methyl tosylates **25** with various amine nucleophiles **26** as depicted in Scheme 1. The (2,3-dihydro-1,4-benzodioxin-2-yl)methyl tosylates **25a**−**e** were synthesized by tosylation of the known (2,3-dihydro-1,4-benzodioxin-2-yl)methanols **27a**−**e**, which were obtained from commercial sources or by preparation as described in the literature.[20−23] Tosylates **25h**,**i** were prepared from the methanols **27h**,**i**, which were in turn obtained from the corresponding esters **28a**,**b** by reduction with $LiAlH_4$. The esters **28a**,**b** were initially prepared as a mixture by literature methods.[24] To separate the two regioisomers, the mixture was converted into the carboxamides **29a**,**b**, which were separated by fractional crystallization and then alcoholized back to the individual esters (Scheme 2).

The remaining tosylates **25f**,**g** were obtained by the synthetic route outlined in Scheme 3. This involved alkylation of the substituted salicylaldehydes **30a**,**b** with (*R*)-glycidyl tosylate to give the ethers **31a**,**b** and then subsequent Baeyer−Villiger oxidation to give the formate esters **32a**,**b**. Alkylations of this type are known to proceed predominantly with retention of configuration at the glycidyl asymmetric center,[25] thus leading to the (*S*)-(2,3-dihydro-1,4-benzodioxin-2-yl)methanols. This stereochemical course was confirmed for the final product **24** by X-ray crystallographic studies[26] which showed it to have the *S*-stereochemistry. All other levorotatory final products are assumed, therefore, to share the *S*-stereochemistry.

Treatment of **32a**,**b** with sodium methoxide in methanol resulted in cleavage of the formate ester and concomitant cyclization, yielding the (2,3-dihydro-1,4-benzodioxin-2-yl)methanols **27f**,**g**. Finally, reaction with tosyl chloride in pyridine gave the tosylates **25f**,**g** in good yield.

The intermediate amines **26a**−**i** were synthesized by the route described in Scheme 4. Reaction of 1-chloro-2,4-dinitrobenzene with isonicotinamide at 95 °C, in the absence of solvent, led to the formation of the pyridinium salt **33**, which on amine exchange[27] gave the pyridinium salts **34a**−**g**. Subsequent reduction of the pyridine ring was accomplished using H_2 on Pd/C, affording piperidines **35a**−**g**. Finally, reduction with $LiAlH_4$ furnished the key amines **26a**−**g**.

Table 1. Structures and Comparative in Vitro and in Vivo Activities for Target and Standard Compounds



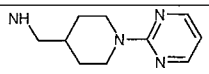
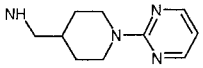
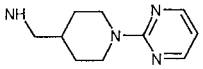
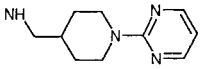
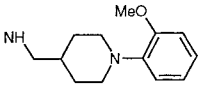
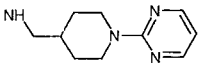
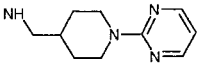
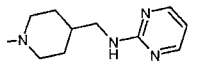
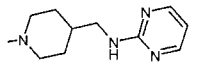
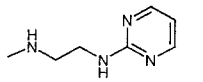
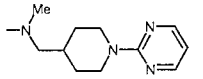
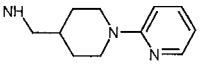
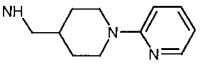
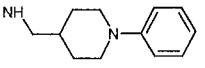
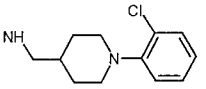
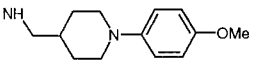
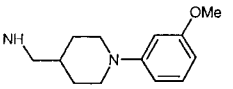
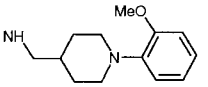
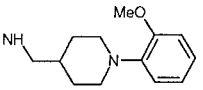
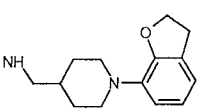
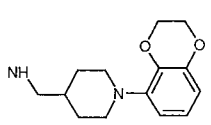
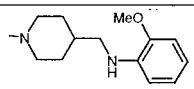
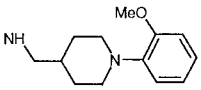
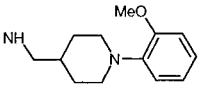
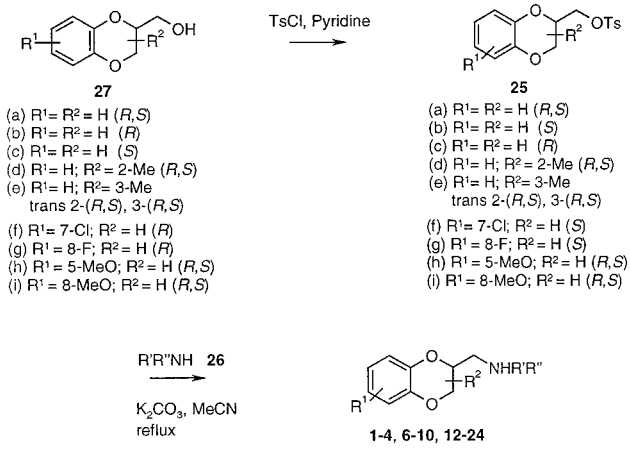
	Compound	R^1	R^2	R^3	R^4	D_2-like[a]	5-HT_{1A}[b]	α_1[b]	AC[c]	CAT[d]
1	(*S*)-(-)		H	H	H	26	2.7 ± .3	18 ± 3	9	44
2	(*R*)-(+)		H	H	H	59	25 ± 1.8	117 ± 19	77%@50 mg/kg	NT
3	(*R*,*S*)		H	H	8-OMe	7.6	0.6 ± .1	2.3 ±.1	2.4	NT
4	(*R*,*S*)		H	H	5-OMe	64	128 ± 14	234 ± 29	NT	NT
5	(*R*,*S*)		H	H	8-OH	25	94%	>500	33	NT
6	(*R*,*S*)		Me	H	H	97	195 ± 23	132 ± 15	NT	NT
7	2-(*R*,*S*)-3-(*R*,*S*)		H	Me	H	113	>500	>500	134	775
8	(-)		H	H	H	6.9	21± 3.3	23 ± 2	NT	NT
9	(+)		H	H	H	112	463 ± 41	208 ± 39	NT	NT
10	(*R*,*S*)		H	H	H	16	3.1 ± 4.2	16 ± 2	6	NT
11	(*S*)		H	H	H	260	85%	348 ± 3	NT	NT
12	(-)		H	H	H	24	98%	98%	23	NT
13	(+)		H	H	H	27	82%	84%	55%@50 mg/kg	NT
14	(*R*,*S*)		H	H	H	39	29 ± 3.3	47 ± 5	39	NT
15	(*R*,*S*)		H	H	H	30	17 ± 2.1	94 ± 16	IA @ 10 mg/kg	NT
16	(*R*,*S*)		H	H	H	36	15 ± 2.4	44 ± 4	11 (duration 2h)	NT
17	(*R*,*S*)		H	H	H	54	7 ± 2	35 ± 4	45% @ 50 mg/kg	NT
18	(*S*)-(-)		H	H	H	26	8 ± 1.9	23 ± 2	7 (duration 3.5 h)	50
19	(*R*)-(+)		H	H	H	64	37 ± 7.6	74 ± 6	38	NT
20	(*R*,*S*)		H	H	H	31	3 ± .3	33 ± 4	18 (duration 2 h)	107
21	(*R*,*S*)		H	H	H	23	18 ± 4.5	38 ± 6	32 (duration 6h)	>200

Table 1 (Continued)

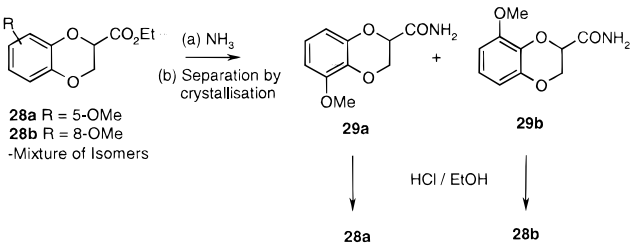
Compound		R^1	R^2	R^3	R^4	D_2-like[a]	5-HT_{1A}[b]	α_1[b]	AC[c]	CAT[d]
22	(*R,S*)		H	H	H	69	20 ± 1.3	20 ± 3	62	NT
23	(*S*)-(-)		H	H	8-F	28	16 ± .2	12 ± 4.7	4 (duration 4h)	23
24	(*S*)-(-)		H	H	7-Cl	44	22 ± 3.7	53 ± 3	5 (duration >6h)	188
haloperidol						2.2	>500	5.7 ± 1.2	0.16	0.44
risperidone						5.1	720 ± 22	1.0 ± .1	0.30	1.75
clozapine						359	415 ± 33	11 ± .9	9.5	33.9
olanzapine						15.7	>500	14 ± 1.2	4.87	6.07

[a] K_i values (nM). Values are from single experiments where dose−response curves were determined with at least six concentrations of compound, and each displacement was measured in triplicate. [b] K_i values (nM) ± SEM. Percentages are for displacement of radioligand at 10^{-6} M. [c] ED_{50} values (mg/kg po) for antagonism of apomorphine-induced climbing in the mouse. Percentages are for reduction in climbing at dose given. Duration is defined by the time at which level of climbing has returned to 50% of control following a dose of 2 × ED_{50}. [d] ED_{50} values (mg/kg ip) for induction of catalepsy in the rat. IA, inactive—causing <50% reduction of climbing at highest dose; NT, not tested.

Scheme 1



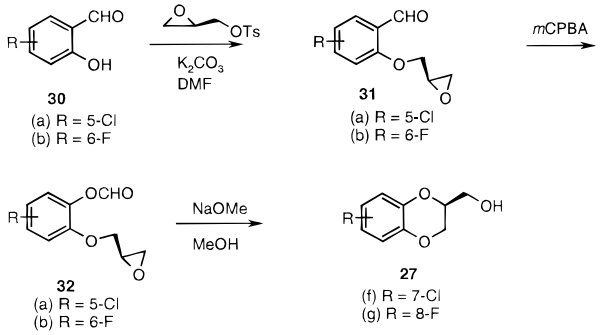
Scheme 2



The amine **26h** was prepared by direct reaction of 2-chloropyridine with 4-(aminomethyl)piperidine **36a**, whereas amine **26i** was prepared from 2-chloropyrimidine and the imine **36b** followed by acid hydrolysis, as depicted in Scheme 4.

In the pyrimidine series, analogues in which the bridging aminomethylpiperidine group was reversed were prepared by reaction of tosylate **25a** with the protected amine **45**, followed by hydrolysis to the primary amine and reaction with 2-chloropyrimidine (Scheme 5). Preparative chiral HPLC then gave enantiomers **8** and **9**. The corresponding 2-methoxyphenyl analogue was prepared by alkylation of ethyl 4-piperidinecarboxylate with 2-(chloromethyl)-2,3-dihydro-1,4-benzodioxin, giving the ester **37**, which on base hydrolysis gave the carboxylic acid **38**. Subsequent mixed anhydride formation with ethyl chloroformate and reaction with 2-methoxyaniline gave the amide **39**, which was reduced with borane-dimethyl sulfide complex to furnish the target compound **22** (Scheme 6).

Scheme 3



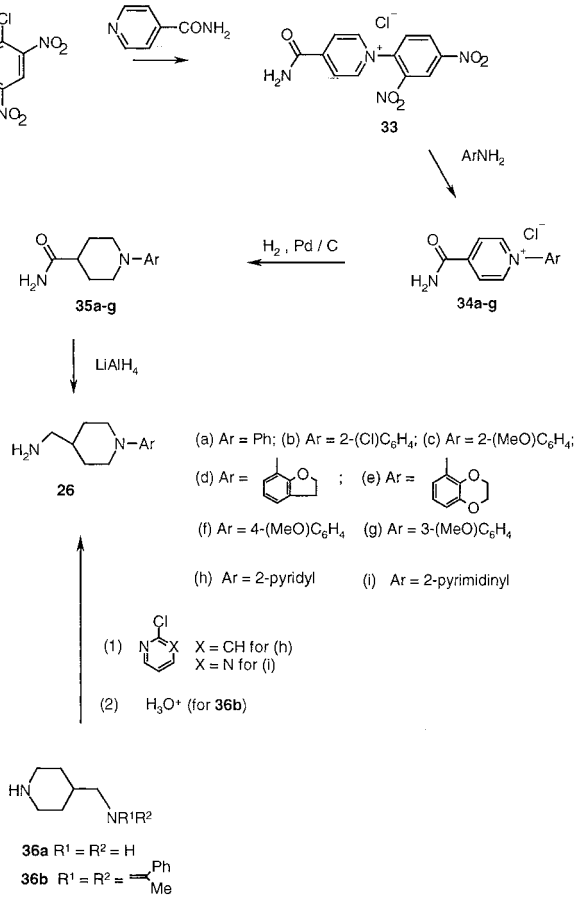
The open-chain analogue **10** was synthesized by the route described in Scheme 7. Reduction of 2,3-dihydro-1,4-benzodioxin-2-carbonitrile with $LiAlH_4$ afforded the methylamine **40** which, when reacted with *N*-(3-bromopropyl)phthalimide, gave intermediate **41**. Subsequent cleavage of the phthalimide group with hydrazine produced the primary amine **42**, which upon reaction with 2-chloropyrimidine gave a mixture of the desired target compound **10** and the bis(2-pyrimidinyl) derivative **43**.

Compound **11** was prepared in 46% yield by direct methylation of **1** using a mixture of sodium formate, formaldehyde, and formic acid. The 8-hydroxy derivative **5** was prepared by hydrolysis of the corresponding 8-tosyloxy compound **49** (itself obtained via ditosylate **48**) using potassium hydroxide in ethanol (Scheme 8).

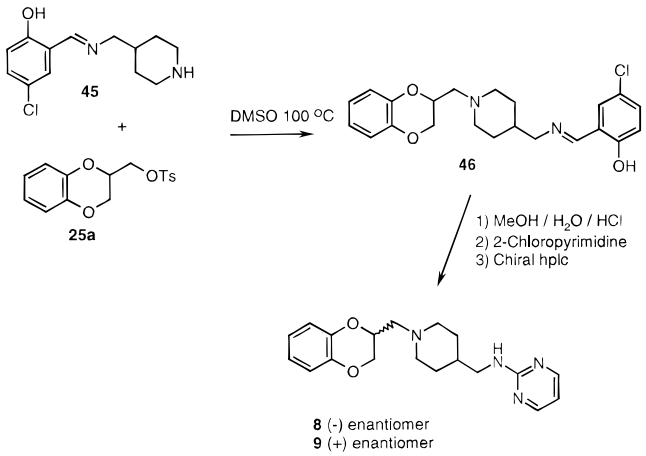
Biological Results

Compound library screening gave the lead *N*-substituted (2,3-dihydro-1,4-benzodioxin-2-yl)methylamine derivative **1** and its enantiomer **2**, originally prepared for another CNS project, which showed moderate to good

Scheme 4



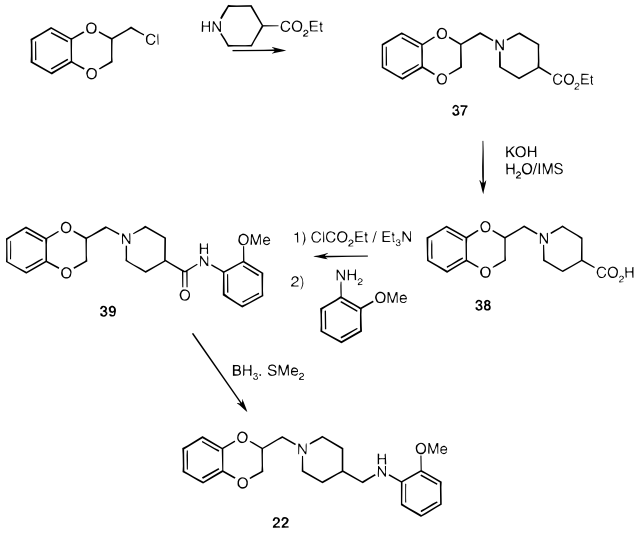
Scheme 5



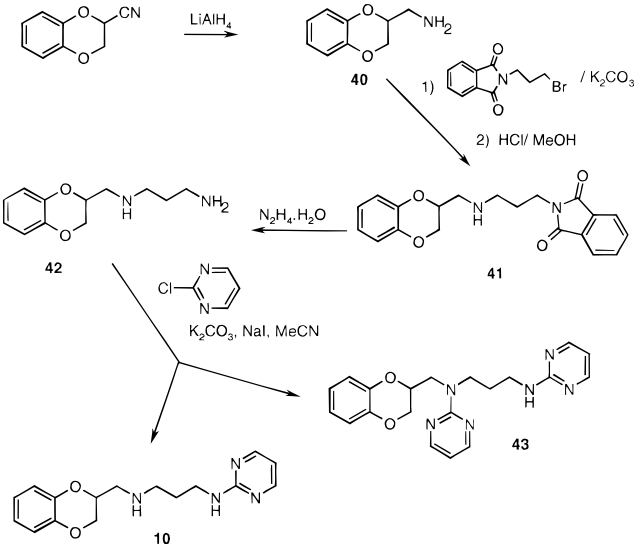
affinities for rat D_2-like and 5-HT_{1A} receptors, with the (−)-isomer **1** being the more active (Table 1). Compound **1** also showed moderate activity in the mouse apomorphine climbing test (AC), a model of antipsychotic activity, having an ED_{50} of 9 mg/kg after oral administration. The (+)-isomer was less active, giving a 77% reduction in climbing at 50 mg/kg.

Further in vivo profiling of **1**, in the two-component amphetamine stereotypy test,[3] showed the compound to be approximately equipotent in antagonizing the effects of amphetamine-induced dopamine release in the limbic and striatal regions of rat brain (ED_{50} values of 2 mg/kg and 1.5 mg/kg, respectively). This indicates that the relative lack of EPS potential shown by **1** (see below)

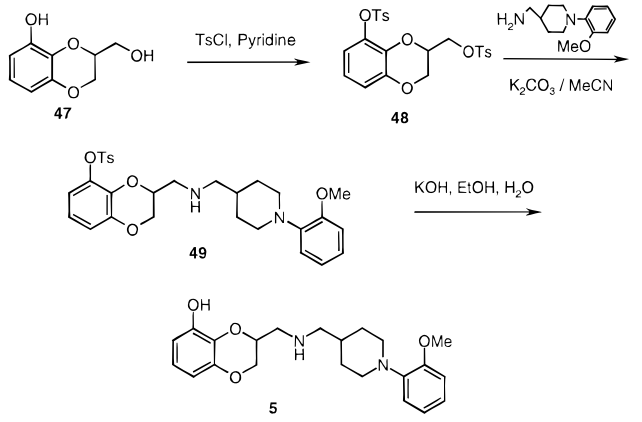
Scheme 6



Scheme 7



Scheme 8



does not result from selective attenuation of postsynaptic dopamine receptor function in the limbic area of the brain.

Compound **1** caused a relatively weak induction of catalepsy[3] in rats, the ED_{50} value (Table 1) being 21 times greater than that for antagonism of the limbic amphetamine-induced locomotion effects. At 30 mg/kg

1 caused a significant 20% reduction in mouse brain levels of the 5-HT precursor 5-HTP, indicating that the compound has agonist activity at 5-HT_{1A} receptors.

As the classical antipsychotic haloperidol, a D_2-like antagonist with little or no 5-HT_{1A} receptor affinity, induces catalepsy in rats with an ED_{50} of only 4 times greater than that in the limbic amphetamine-induced locomotion test, the results for **1** strongly suggested that combining D_2-like antagonism with 5-HT_{1A} (partial) agonism in a single molecule would give effective antipsychotic compounds with a reduced propensity to induce extrapyramidal side effects.

The relatively high α_1 affinity of **1** was seen as a shortcoming, as this would be likely to lead to unwanted cardiovascular effects, including postural hypotension. In addition to reduced α_1 affinity, greater potency in vivo and a further reduction in cataleptic potential were also considered desirable. A further requirement for a development compound was that it should have the potential for once or twice daily dosing in man. As a predictor of this, greater than 6 h duration (see Table 1 for definition) would be required in the AC test. Therefore, further analogues of compound **1** were prepared in order to address these concerns.

Substitution in the aromatic ring of the dihydrobenzodioxin system was not explored extensively in this pyrimidine-containing subseries, although 5- and 8-methoxy substituents were found to have opposite effects on receptor affinity. The racemic 8-methoxy compound **3** had significantly higher affinity for D_2-like, 5-HT_{1A}, and α_1 receptors than **1**, and it was correspondingly more active in AC. The 5-methoxy compound **4**, in contrast, had much reduced affinity for these three receptors. The 8-hydroxy compound **5** also had good affinity for D_2-like and 5-HT_{1A} receptors, but was only weakly active in AC.

Introduction of a methyl substituent into the 2- or 3-position of the dihydrobenzodioxin system, as in compounds **6** and **7**, led to significant loss of receptor affinities.

Reversal of the bridging 4-(aminomethyl)piperidine grouping found in **1** gave the enantiomers **8** and **9**, which showed receptor binding profiles similar to **1** and **2**, respectively. Incorporation of an acyclic bridge, as in the racemate **10**, also led to activity similar to that of **1**, both in receptor affinities and in AC activity.

N-Methylation of **1**, to give the tertiary amine **11**, led to significant loss of receptor affinities.

It seemed at this point that neither substituent variation in the benzodioxin ring nor alternative configurations of the bridging portion of the side chain would give us the potency and selectivity we required. Alternatives to the 2-pyrimidinyl grouping were therefore explored, in the form of the 2-pyridyl containing enantiomers **12** and **13**, and the phenyl and 2-chlorophenyl containing racemates **14** and **15**. Although receptor affinities were maintained, activity in AC was moderate at best. It was not clear which of many potential factors, including degree of functional activity at D_2 and 5-HT_{1A} receptors and bioavailability, were adversely affecting the in vivo activity of these compounds, but it seemed worthwhile to try to mimic the H-bond acceptor properties of the pyrimidines with alkoxyphenyl groups. Thus, the 4-, 3-, and 2-methoxyphenyl analogues **16**−**19** were found to show similar binding profiles, with the 4- and 2-isomers showing significant in vivo activity. The (−)-enantiomer **18** of the 2-methoxy compound showed acceptable potency and duration of action in the AC test. This compound also showed good activity in antagonizing limbic amphetamine-induced stereotypy (ED_{50} 4.4 mg/kg), combined with only a moderate ability to induce catalepsy. The corresponding (+)-isomer **19** was again less active both in vitro and in vivo.

A concern with the methoxy substituted compounds was their propensity for metabolic demethylation, which could have adverse effects on the compound's pharmacokinetics. Investigation of heterocyclic alternatives to 2-methoxyphenyl, which may be more stable metabolically, led to the 2,3-dihydrobenzofuran **20** and the 2,3-dihydro-1,4-benzodioxin **21**. Disappointingly, both had inferior activity in AC.

Reversal of the 4-(aminomethyl)piperidyl bridging group gave racemate **22**, which had significantly reduced activity compared to compound **18**, consistent with the pyrimidine series.

At the time of this work, the only suggestions in the literature of how to obtain selectivity over α_1 activity in this class of compounds was through 7,8-benzofusion.[14] Therefore, substitution in these two positions was explored in order to determine whether selectivity over α_1 could be improved. As reported above, incorporation of an 8-methoxy substituent into the pyrimidine subseries actually increased affinity for α_1 receptors. A similar effect was seen in the 2-methoxyphenyl containing subseries, where introduction of an 8-fluoro substituent (compound **23**) led to good activity in vitro and in vivo. The relatively high α_1 affinity of this compound, however, precluded its further development, despite its low cataleptic activity. Introduction of a 7-chloro substituent (compound **24**) was found to give a more useful improvement, as affinity for rat α_1 receptors was not too high. Indeed, when human receptors were examined, selectivity for D_2, D_3, and 5-HT_{1A} receptors over α_1 receptors was much improved (see later). In addition, the duration of action of **24** in AC was now greater than 6 h, and the ED_{50} for catalepsy was increased to 188 mg/kg, 100-fold greater than its ED_{50} for antagonism of limbic amphetamine-induced locomotor activity, 1.8 mg/kg. The corresponding ratios for haloperidol, clozapine, olanzapine, and risperidone are 4, 9, 10, and 22, respectively (Figure 1). Because compound **24** had a desirable receptor binding profile and showed good in vivo activity, it was selected for further evaluation.

Against a panel of other neurotransmitter receptors, including 5-HT_{1A} and α_1 receptors from postmortem human brain (Table 2), **24** was found to have high affinity only for hD_{2L}, hD_3, and h5-HT_{1A} subtypes, with moderate affinity being shown for $hD_{4.2}$, 5-HT_7, and 5-HT_{2C}. The encouraging separation between affinities shown by **24** for human D_2-like and 5-HT_{1A} versus α_1 receptors suggests postural hypotension is unlikely to be significant in clinical studies with this compound.

Compound **24** increased levels of the dopamine metabolites DOPAC and HVA in the rat brain with ED_{200} of 2 mg/kg for DOPAC in both limbic and striatal tissue, thereby demonstrating functional dopamine antagonism. Also in rat brain, **24** reduced levels of the 5-HT

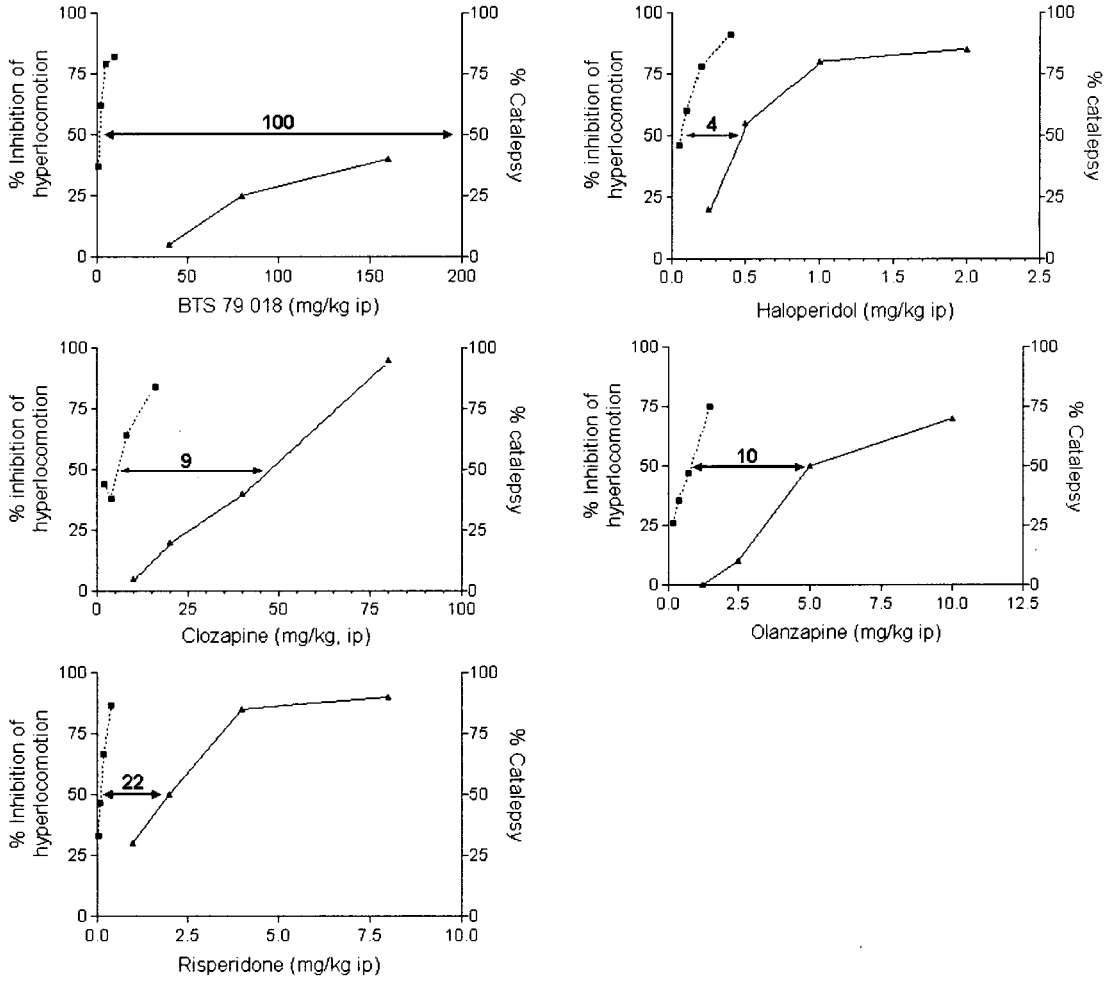


Figure 1. Comparison of the effects on catalepsy and amphetamine hyperlocomotion for BTS 79 018 (**24**) and reference compounds. Key: percent catalepsy (▲−▲), percent inhibition of amphetamine hyperlocomotion (■···■), therapeutic index (extrapolated for **24**) (↔). Compound **24**, like the comparator antipsychotics, potently inhibits amphetamine-induced hyperlocomotion in rats after ip administration; but unlike the other drugs, it shows a very low propensity to induce catalepsy. The ratio of the ED_{50} of a drug to attenuate amphetamine-induced hyperlocomotion (highly predictive of antipsychotic efficacy) to its ED_{50} to induce catalepsy (highly predictive of movement disorder liability) provides a therapeutic index for the drug. The ratios were calculated from the following ED_{50} values (mg/kg with 90% confidence limits). Compound **24**: hyperlocomotion 1.8 (0.78−3.9); catalepsy 190 (79−450). Clozapine: hyperlocomotion 3.9 (1.7−8.8); catalepsy 34 (19−160). Haloperidol: hyperlocomotion 0.08 (0.03−0.22); catalepsy 0.44 (0.15−1.4). Olanzapine: hyperlocomotion 0.63 (0.28−1.4); catalepsy 6.1 (3.1−12). Risperidone: hyperlocomotion 0.078 (0.029−0.21); catalepsy 1.8 (0.82−3.8). Compound **24** clearly has the best therapeutic index of any drug tested; as expected haloperidol has the worst (**24** ≫ risperidone > olanzapine > clozapine > haloperidol).

Table 2. Further Receptor Binding Affinities for **24** (BTS 79 018)[a]

hD_1	hD_{2L}	hD_3	$hD_{4.2}$	hD_5	$h5\text{-}HT_{1A}$
>1000	13.2 ± 3.4	1.6 ± .09	70.9 ± 12.3	>1000	3.6 ± 0.5
$h\alpha_1$	α_{2D}	$5\text{-}HT_{1B}$	$5\text{-}HT_{2A}$	$5\text{-}HT_{2C}$	$5\text{-}HT_3$
181 ± 18	>500	>1000	>500	58 ± 3.9	>1000
$5\text{-}HT_4$	$5\text{-}HT_6$	$5\text{-}HT_7$	MCB	H_1	
>1000	>500	27 ± 5	>1000	>1000	

[a] K_i values (nM) ± SEM.

precursor 5-HTP by 25% at a dose of 30 mg/kg, consistent with an agonist effect at $5\text{-}HT_{1A}$ receptors. Compound **24** showed no effect in the Porsolt test at 100 mg/kg, but reversed the effect of 8-OH-DPAT in this test, with an ED_{50} of 11.9 mg/kg. Taken together these data demonstrate in vivo $5\text{-}HT_{1A}$ partial agonist activity.

Compound **24**, BTS 79 018, demonstrated the requisite activity in rodent models of antipsychotic activity, together with a predicted low propensity to cause extrapyramidal and cardiovascular side effects. It has, therefore, been selected for clinical studies to determine its potential as a novel atypical antipsychotic therapeutic agent.

Conclusion

Synthesis of analogues of the lead *N*-substituted (2,3-dihydro-1,4-benzodioxin-2-yl)methylamine derivative **1** resulted in the optimization of in vitro receptor binding activity and in vivo activity in rodent models of psychosis, leading to compound **24**, which had the required

good affinities for human D_2/D_3 and 5-HT_{1A} receptors, with significantly lower affinity for human α_1 adrenoceptors and rat H_1 and muscarinic receptors. In rodents, **24** showed functional D_2-like antagonism and 5-HT_{1A} partial agonism. After oral dosing most analogues showed good activity in rodent antipsychotic tests. This was combined in compound **24** with a long duration of action and very little potential to cause EPS, as measured by its ability to induce catalepsy in rats only at very high doses compared to doses effective in antipsychotic models. In light of this promising profile of activity, **24** has been selected for clinical investigation as a novel antipsychotic agent with a predicted low propensity to cause EPS.

Experimental Section

Chemistry. Proton magnetic resonance spectra were recorded on Bruker AM 360 or AC 250 spectrometers and are reported in ppm on the δ scale from internal tetramethylsilane. Infrared spectra were obtained using a Mattson-Unicam 3000 FTIR spectrometer. Elemental analyses were determined by the Physical Chemistry Department (Knoll Pharmaceuticals, Nottingham). Where analyses are indicated by the symbols of the elements, analytical results were within ±0.4% of the theoretical values. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Thin-layer chromatography was performed using Merck silica gel 60 F_{254} plates. Flash column chromatography was carried out on silica 60 A. The term "dried" refers to use of anhydrous magnesium or sodium sulfate. A general description of the synthetic procedure is given where applicable.

Preparation of (2,3-Dihydro-1,4-benzodioxin-2-yl)-methyl 4-Toluenesulfonates 25a−i, 48. General Procedure for the 4-Toluenesulfonylation of (2,3-Dihydro-1,4-benzodioxin-2-yl)methanols 27a−i, 47, e.g., (*R*)-(8-Fluoro-2,3-dihydro-1,4-benzodioxin-2-yl)methyl4-Toluenesulfonate 25. A stirred solution of (*S*)-(8-fluoro-2,3-dihydro-1,4-benzodioxin-2-yl)methanol **27g** (3.85 g, 20.9 mmol) in pyridine (50 mL) was treated with 4-toluenesulfonyl chloride (4.10 g, 21.6 mmol), and the resulting solution was stirred overnight at room temperature. The reaction mixture was poured into excess hydrochloric acid (5 M) and extracted with ethyl acetate. The combined extracts were washed with brine, dried, and concentrated under reduced pressure to give **25g** (4.10 g, 58%): ^{1}H NMR (DMSO-d_6) δ 7.77 (d, J = 8.3 Hz, 2H, 4-toluenesulfonyl *m*-H), 7.42 (d, J = 8.3 Hz, 2H, 4-toluenesulfonyl *o*-H), 6.75 (m, 2H, aromatic H), 6.58 (m, 1H, aromatic H), 4.48 (m, 1H), 4.25 (m, 3H), 4.04 (m, 1H), and 2.38 (s, 3H, Me).

Preparation of (2,3-Dihydro-1,4-benzodioxin-2-yl)-methanols 27b−i, 47. 2,3-Dihydro-1,4-benzodioxin-2-ylmethanols **27b**,[20] **27c**,[20] **27d**,[21] **27e**,[22] and **47**[23] were synthesized according to literature procedures. Compound **27a** was purchased from a commercial supplier.

(*S*)-(7-Chloro-2,3-dihydro-1,4-benzodioxin-2-yl)methanol (27f). A stirred solution of 5-chlorosalicylaldehyde **30a** (8.92 g, 57.0 mmol) in dry dimethylformamide (200 mL) was treated with potassium carbonate (7.87 g, 57.0 mmol) and (*R*)-glycidyl 4-toluenesulfonylate (13.0 g, 57.0 mmol) and the mixture heated with stirring at 60 °C for 5 h. The mixture was then cooled and water (200 mL) added. The resulting mixture was extracted with ether (3 × 150 mL), and the combined extracts were washed with brine, dried, and concentrated under reduced pressure to give an oil. The residue was purified by flash column chromatography on silica eluting with a 25:1 mixture of petroleum ether (bp 40−60 °C) and ethyl acetate and then a 10:1 mixture of petroleum ether (bp 40−60 °C) and ethyl acetate to give (*R*)-5-chloro-2-(2,3-epoxypropoxy)benzaldehyde **31a** (8.10 g, 67%) as a colorless oil which solidified on standing: ^{1}H NMR ($CDCl_3$) δ 10.45 (s, 1H, CHO), 7.80 (d, J = 2.7 Hz, 1H, aromatic H), 7.48 (dd, J = 8.9, 2.7 Hz, 1H, aromatic H), 6.97 (d, J = 8.9 Hz, 1H, aromatic H), 4.40 (dd, J = 11.1, 2.7 Hz, 1H), 4.06 (m, 1H), 3.41 (m, 1H), 2.96 (t, J = 4.6 Hz, 1H), and 2.78 (m, 1H).

3-Chloroperoxybenzoic acid (86%; 9.20 g, 45.87 mmol) was added to a stirred solution of **31a** (8.10 g, 38.11 mmol) in dichloromethane (100 mL), and the mixture was heated under reflux for 20 h and then cooled to room temperature. The resulting solid was filtered off and washed with dichloromethane. The filtrate was washed with saturated aqueous sodium metabisulfite solution (100 mL), saturated aqueous sodium bicarbonate solution (2 × 100 mL), and brine (100 mL), dried, and concentrated under reduced pressure to give (*R*)-5-chloro-2-(2,3-epoxypropoxy)phenyl formate **32a (**7.98 g, 92%) as an orange oil: IR (film) 1745 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H, OCHO), 7.19 (dd, J = 8.8, 2.6 Hz, 1H, aromatic H), 7.12 (d, J = 2.6 Hz, 1H, aromatic H), 6.96 (d, J = 8.8 Hz, 1H, aromatic H), 4.26 (dd, J = 8.0, 2.9 Hz, 1H), 3.95 (m, 1H), 3.30 (m, 1H), 2.88 (t, J = 4.7 Hz, 1H), and 2.72 (m, 1H).

A solution of the epoxide **32a** (5.67 g, 24.81 mmol) in methanol (80 mL) was added slowly to a stirred solution of sodium methoxide, prepared from sodium (0.65 g, 28.26 mmol) and methanol (50 mL) under nitrogen. The resulting solution was stirred overnight at room temperature and concentrated under reduced pressure, and the residue was partitioned between ether and water. The ether layer was then washed with brine, dried, and concentrated under reduced pressure to give (*S*)-(7-chloro-2,3-dihydro-1,4-benzodioxin-2-yl)methanol **27f** (4.40 g, 88%) as a pale-yellow solid, mp 61−62 °C: ^{1}H NMR ($CDCl_3$) δ 6.90 (d, J = 1.5 Hz, 1H, aromatic H), 6.80 (s, 2H, aromatic H), 4.24 (m, 2H), 4.11 (m, 1H), 3.86 (m, 2H), and 1.96 (t, J = 6.3 Hz, 1H, O*H*).

(*S*)-(8-Fluoro-2,3-dihydro-1,4-benzodioxin-2-yl)methanol (27g). Alcohol **27g** was prepared following the same procedure as that for **27f** starting from 6-fluorosalicylaldehyde **30b**, which was synthesized by boron tribromide demethylation of 6-fluoro-2-methoxybenzaldehyde, which in turn was prepared by lithiation of 3-fluoroanisole and subsequent reaction with dimethylformamide following the procedure of Bridges et al.[28]

(*R*,*S*)-(5-Methoxy-2,3-dihydro-1,4-benzodioxin-2-yl)-methanol (27h) and (*R*,*S*)-(8-Methoxy-2,3-dihydro-1,4-benzodioxin-2-yl)methanol (27i). Reaction of 3-methoxycatechol (60.2 g, 0.42 mol) with ethyl 2,3-dibromopropionate (90.0 g, 0.50 mol) and potassium carbonate (129.0 g, 0.94 mol) in acetone, using the literature conditions,[24] gave a mixture of 5-methoxy and 8-methoxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid ethyl esters **28a** and **28b** (83.8 g, 84%) which, by GC analysis, were in the approximate ratio of 2:1. A portion of the mixture (65.0 g, 0.27 mol) in absolute ethanol (160 mL) was treated with aqueous ammonia solution (SG 0.880; 500 mL) and the mixture stirred at room temperature for 90 min. The resulting white solid was collected by filtration, washed with a little water, and dried to give a mixture of the two carboxamides **29a** and **29b** (43.45 g, 77%). The mixture was then heated in boiling absolute ethanol (400 mL), and the insoluble material was collected by filtration and then once again heated in boiling absolute ethanol (25 mL). The resulting white solid was collected by filtration, washed with ethanol (20 mL), and dried to give **29a** (18.55 g, 33%) as a white solid, mp 196−198 °C; 97.3% pure by HPLC (S5 C8, MeCN/TEAF buffer 20:80, λ = 210 nm).

The filtrates from all of the ethanol digestions were combined and concentrated under reduced pressure. The residue was then purified by preparative HPLC (HP 1040M, sherisorb 5 μm C8, MeCN/TEAF buffer 20:80, λ = 205 nm) to give **29b** (8.10 g, 14%) as a white solid, mp 184−187 °C.

A stirred solution of the amide **29a** (18.40 g, 0.088 mol) in absolute ethanol (330 mL) was saturated with gaseous hydrogen chloride, and the mixture was heated under reflux for 16 h. The cooled reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was redissolved in ether, washed successively with water, 1 M aqueous sodium hydroxide solution, and water, dried, and concentrated under reduced pressure to give **28a** (18.55 g, 88%) as an oil, 97.5% pure by GC (3 ft, 10% OV-7).

A solution of the ester **28a** (18.54 g, 0.077 mol) in dry ether (100 mL) was added dropwise to a stirred suspension of lithium aluminum hydride (2.0 g, 0.053 mol) in dry ether (150 mL) while the temperature of the reaction was maintained between 10 and 20 °C. The mixture was stirred at room temperature for 2 h and cooled to 5 °C, and then water (5 mL) was added cautiously. Aqueous sodium hydroxide solution (1 M; 12 mL) and water (12 mL) were then added, and the mixture was filtered through Celite. The filtrate was extracted with ether, and the combined extracts were washed with water, dried, and concentrated under reduced pressure to give (*R*,*S*)-(5-methoxy-2,3-dihydro-1,4-benzodioxin-2-yl)methanol **27h** (12.50 g, 79%).

The above procedure was repeated for **29b** (8.0 g, 0.038 mol) giving **27i** (3.25 g, 36%), 95.69% pure by GC (OV-7).

General Procedure for the Preparation of the Amines 26a−g. 1-(1-Phenyl-4-piperidyl)methylamine (26a). A mixture of 1-chloro-2,4-dinitrobenzene (400 g, 1.98 mol) and isonicotinamide (200 g, 1.64 mol) was heated at 95 °C for 1 h. The resulting solid material was cooled, collected by filtration, and washed with a 10:1 solution of ether and methanol. The solid was then triturated with hot methanol (1000 mL), collected, and dried to give 4-carbamoyl-1-(2,4-dinitrophenyl)-pyridinium chloride **33** (449.1 g, 84%) as a cream solid, mp 233−36 °C: IR 1687 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.58 (d, J = 6.8 Hz, 2H), 9.13 (d, J = 2.5 Hz, 1H, aromatic H), 9.05 (br s, 1H, NH), 9.01 (dd, J = 8.7, 2.5 Hz, 1H, aromatic H), 8.75 (d, J = 6.8 Hz, 2H, aromatic H), and 8.44 (d, J = 8.7 Hz, 2H, aromatic H).

A mixture of **33** (50.0 g, 0.15 mol) and aniline (35.60 g, 0.38 mol) in methanol (1000 mL) was stirred at room temperature for 48 h. The resulting suspension was heated at 50 °C for 1 h, cooled, and concentrated under reduced pressure. The solid residue was triturated with acetone (2 × 1000 mL), filtered, and dried to give 4-carbamoyl-1-phenylpyridinium chloride **34a (**33.24 g, 92%) as an off-white solid, mp 290−292 °C: IR 1681 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.56 (d, J = 6.9 Hz, 2H, aromatic H), 9.12 (br s, 1H, NH), 8.67 (d, J = 6.9 Hz, 2H, aromatic H), 8.41 (br s, 1H, NH), 7.92 (m, 2H, aromatic H), and 7.76 (m, 3H, aromatic H). Anal. ($C_{12}H_{11}ClN_2O$) C, H, Cl, N.

A solution of the pyridinium salt **34a** (10.0 g, 42.64 mmol) in ethanol (250 mL) was hydrogenated at atmospheric pressure using hydrogen over a 10% palladium upon carbon catalyst (1.0 g). The catalyst was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure to give 1-phenylpiperidine-4-carboxamide hydrochloride (**35a) (**8.79 g, 86%) as a solid: IR 1679 cm^{-1}.

The above amide hydrochloride **35a** (1.50 g, 6.24 mmol) was added portionwise to a stirred suspension of lithium aluminum hydride (0.50 g, 13.15 mmol) in dry tetrahydrofuran (100 mL), and the mixture was stirred at room temperature for 2 h and then at reflux for 2 h. The mixture was cooled, and water (0.5 mL) was carefully added. Concentrated aqueous sodium hydroxide solution (12.5 M; 0.5 mL) was then added, and the resulting precipitate was filtered through Celite. The filtrate was then dried and concentrated under reduced pressure to give 1-(1-phenyl-4-piperidyl)methylamine **26a (**1.15 g, 97%) as an oil: ^1H NMR ($CDCl_3$) δ 7.23 (m, 2H, aromatic H), 6.96 (d, J = 7.0 Hz, 2H, aromatic H), 6.82 (br t, J = 7.0 Hz, 1H, aromatic H), 3.70 (br d, J = 12.6 Hz, 2H), 2.63 (m, 4H), 1.81 (br d, J = 9.8 Hz, 2H), and 1.39 (m, 5H).

The remaining amines **(26b−g)** were synthesized in the same manner. 5-Amino-2,3-dihydro-1,4-benzodioxin (precursor to **26e**) was prepared according to the literature method,[29] and 7-amino-2,3-dihydrobenzo[*b*]furan (precursor to **26d**) was prepared by Raney nickel reduction of the corresponding known 7-nitro-2,3-dihydrobenzo[*b*]furan.[30]

Preparation of the Amines (26h−i). 4-(Aminomethyl)-1-(2-pyrimidinyl)piperidine (26i). Acetophenone (24.0 g, 0.2 mol) and 4-toluenesulfonic acid (0.40 g, 2.32 mmol) were added to a stirred solution of 4-(aminomethyl)piperidine (22.8 g, 0.2 mol) in dry toluene (200 mL), and the mixture was heated under reflux for 5 h under a Dean and Stark apparatus to give intermediate **36b**, which was not isolated. 2-Chloropyrimidine (22.9 g, 0.2 mol) and triethylamine (14 mL) were added to the resulting solution, and the mixture was heated at 95 °C with stirring for 18 h. The cooled reaction mixture was extracted with hydrochloric acid (5 M; 2 × 200 mL), and the combined extracts were heated at 95 °C for 6 h. The resulting cooled acidic solution was washed with ether and then basified with 5 M aqueous sodium hydroxide solution. The basic solution was extracted with ether, and the extracts were dried and concentrated under reduced pressure to leave a brown oil. The basic aqueous solution was also concentrated to dryness, and the resulting solid mass was extracted with ether. The combined extracts were dried and concentrated under reduced pressure to afford a brown oil. The combined brown oils were dissolved in ether and saturated with dry hydrogen chloride gas. The solid obtained was recrystallized from industrial methylated spirit to yield 4-(aminomethyl)-1-(2-pyrimidinyl)-piperidine 1.6 hydrochloride **26i** (16.50 g, 66%) as a yellow solid, mp 245−248 °C: ^1H NMR (DMSO-d_6) δ 8.43 (d, J = 4.9 Hz, 2H, pyrimidine 4,6-H), 8.24 (br s, 2H, NH_2), 6.72 (t, J = 4.9 Hz, 1H, pyrimidine 5-H), 4.66 (br d, J = 13.3 Hz, 2H), 2.98 (br t, J = 12.2 Hz, 2H), 2.69 (m, 2H), 1.92 (m, 1H), 1.83 (br d, J = 13.3 Hz, 2H), and 1.17 (dq, J = 12.2, 4.0 Hz, 2H). Anal. ($C_{10}H_{16}N_4$·1.6HCl) C, H, N, Cl.

The amine **26h** was prepared in a similar manner by direct reaction of 4-(aminomethyl)piperidine with 2-chloropyridine and sodium carbonate in *iso*-amyl alcohol.

General Procedure for Alkylation of Amines 26a−i by the (2,3-Dihydro-1,4-benzodioxin-2-ylmethyl 4-Toluenesulfonates 25a−i, 48, e.g., Preparation of *N*-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)-1-(1-phenylpiperid-4-yl)methylamine Dihydrochloride (14). A stirred mixture of the 4-toluenesulfonylate **25a** (10.10 g, 0.032 mol), the amine **26a** (6.0 g, 0.032 mol), and potassium carbonate (15.0 g, 0.11 mol) in acetonitrile (200 mL) was heated under reflux for 72 h and then cooled to room temperature. The resulting mixture was then filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and purified by elution through a silica pad, using ethyl acetate as eluant, to give an oil after evaporation of solvent. The oil was dissolved in ether and saturated with hydrogen chloride. The resulting solid was collected by filtration and triturated with a 25:1 solution of ethyl acetate in methanol to give *N*-(2,3-dihydro-1,4-benzodioxin-2-yl)-1-(phenylpiperid-4-yl)methylamine dihydrochloride **(14)** (5.64 g, 43%), mp 278−280 °C: ^1H NMR (DMSO-d_6) δ 9.75 (br s, 1H, HCl), 9.55 (br s, 1H, HCl), 7.87 (br s, 2H aromatic H), 7.50 (m, 3H aromatic H), 6.89 (m, 4H aromatic H), 4.81 (m, 1H), 4.44 (dd, J = 11.7, 2.3 Hz, 1H), 4.08 (dd, J = 11.7, 6.6 Hz, 1H), 3.30 (m, 8H) and 2.09 (m, 5 H). Anal. ($C_{21}H_{26}N_2O_2$·2HCl) C, H, Cl, N.

Compounds **1**−**4**, **6**−**7**, **12**−**13**, **15**−**21**, **23**−**24**, and **49** were prepared by similar procedures.

For chiral compounds **1** and **18**, the (*R*)-4-toluenesulfonylate **25b**, prepared from the (*S*)-alcohol **27b**, was used in the reaction with the appropriate amine. Similarly, for **2** and **19** the (*S*)-4-toluenesulfonylate **25c**, prepared from the (*R*)-alcohol **27b**, was employed. The enantiomers **12** and **13** were separated by chiral HPLC (Chiralcel OC column eluting with hexane/ethanol 1:1 at 5 then 10 mL/min). Specific rotations and, where determined, HPLC derived enantiomeric excesses for all chiral target compounds are given below:

1 $[\alpha]_D$ −51.2° (c = 1.0, MeOH) 85.4% e.e. (Chiralcel OC, hexane/ethanol 1:1); **2** $[\alpha]_D$ +49.5° (c = 1.0, MeOH) 84.8% e.e. (Chiralcel OC, hexane/ethanol 1:1); **8** $[\alpha]_D$ −63.0° (c = 1.0, MeOH) 100% e.e.; **9** $[\alpha]_D$ +63.0° (c = 1.0, MeOH) 100% e.e.; **12** $[\alpha]_D$ −63.0° (c = 1.0, MeOH) 100% e.e. (Chiralcel OC, hexane/ethanol 1:1); **13** $[\alpha]_D$ +63.0° (c = 1.0, MeOH) 100% e.e. (Chiralcel OC, hexane/ethanol 1:1); **18** $[\alpha]_D$ −27.0° (c = 1.0, CH_2Cl_2); **19** $[\alpha]_D$ +25.3° (c = 1.0, CH_2Cl_2); **23** $[\alpha]_D$ −49.2° (c = 1.0, MeOH); **24** $[\alpha]_D$ −42.6° (c = 0.5, EtOH) 99.8% e.e. (Chiralcel OD, isohexane/2-propanol 1:1 containing 0.1% diethylamine).

***N*-(8-Hydroxy-2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-1-[1-(2-methoxyphenyl)piperid-4-yl]methylamine (5).** A solution of potassium hydroxide (1.0 g, 17.8 mmol) in a mixture of water (19.7 mL) and ethanol (19.7 mL) was added portion-

wise to 2-({1-[1-(2-methoxyphenyl)piperid-4-yl]methylamino}-methyl)-2,3-dihydro-1,4-benzodioxin-8-yl 4-toluenesulfonate **49** (0.59 g, 0.93 mmol), and the mixture was heated under reflux with stirring for 2.5 h. The cooled mixture was neutralized with glacial acetic acid and extracted with ether (3 × 100 mL). The combined extracts were left to stand overnight, and the resulting white solid was collected by filtration and dried to give *N*-(8-hydroxy-2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-1-[1-(2-methoxyphenyl)piperid-4-yl]methylamine **5** (0.20 g, 64%), mp 116−119 °C: IR 3552 cm $^{-1}$; ^{1}H NMR (DMSO-d_6) δ 9.00 (br s, 1H, OH), 6.88 (m, 4H, aromatic H), 6.58 (t, J = 8.1 Hz, 1H, aromatic H), 6.36 (dd, J = 8.0, 1.5 Hz, 1H, aromatic H), 6.31 (dd, J = 8.0, 1.5 Hz, 1H, aromatic H), 4.30 (dd, J = 11.2, 2.1 Hz, 1H), 4.15 (m, 1H), 3.91 (dd, J = 11.2, 7.6 Hz, 1H), 3.76 (s, 3H, OMe), 2.77 (m, 2H), 1.78 (br d, J = 12.1 Hz, 2H), 1.51 (m, 1H), and 1.31 (m, 2H)—remaining signals are obscured by water and DMSO peaks. Anal. ($C_{22}H_{28}N_2O_4$·3.5H_2O) C, H, N.

***N*-(2-Pyrimidinyl)-3-[1-(2,3-dihydro-1,4-benzodioxin-2-yl]methylamino)propylamine (10).** A suspension of 2,3-dihydro-1,4-benzodioxin-2-carbonitrile[31] (15.0 g, 0.093 mol) in dry ether (50 mL) was added portionwise to a stirred suspension of lithium aluminum hydride (5.25 g, 0.14 mol) in dry ether (100 mL). The mixture was then stirred and heated under reflux for 1 h. The cooled mixture was carefully treated with water (7 mL), aqueous sodium hydroxide solution (2 M; 7 mL), and then water (30 mL). The resulting mixture was filtered, and the solid was washed with ethyl acetate and water. The filtrate was separated, and the aqueous phase was extracted with ethyl acetate. The extracts and the original organic phase were combined, dried, and concentrated under reduced pressure to give 1-(2,3-dihydro-1,4-benzodioxan-2-yl)-methylamine **40** (12.20 g, 80%) as an orange oil, 98% pure by GC (OV-7).

A mixture of the product **40** from the previous reaction (6.0 g, 0.036 mol), *N*-(3-bromopropyl)phthalimide (9.75 g, 0.036 mol), and potassium carbonate (9.94 g, 0.072 mol) in dry acetonitrile (100 mL) was heated under reflux with stirring for 24 h. The mixture was cooled and filtered, and the filtrate was concentrated under reduced pressure to give an orange gum. The gum was then stirred in hydrochloric acid (5 M; 100 mL) to give a brown solid which was recrystallized from methanol to give the phthalimide hydrochloride **41** (7.10 g, 56%) as a cream solid, mp 225−300 °C. Anal. ($C_{20}H_{21}ClN_2O_4$) H, N; C: calcd, 61.8; found, 61.2.

Hydrazine hydrate (0.46 mL, 9.30 mmol) was added to a stirred suspension of the phthalimide **41** (3.00 g, 7.7 mmol) in methanol (50 mL), and the mixture was heated under reflux for 1 h. Concentrated hydrochloric acid (4 drops) was then added, and the mixture was stirred at room temperature for 1 h. A colorless solid which separated was removed by filtration, and the filtrate was concentrated under reduced pressure. The resulting residue was triturated with ether to give the propylamine dihydrochloride **42** (2.00 g, 88%) as a cream solid, mp 235−240 °C; 97.3% pure by HPLC (Asahipak ODP-50, MeCN/TEAF buffer 5:95, λ = 249 nm).

A stirred mixture of the propylamine **42** (1.61 g, 7.25 mmol), potassium carbonate (2.00 g, 14.5 mmol), 2-chloropyrimidine (0.87 g, 7.25 mmol), and sodium iodide (0.1 g) in dry acetonitrile (60 mL) was heated at reflux under nitrogen for 5 days. The cooled mixture was filtered, and the filtrate was concentrated under reduced pressure to give a viscous oil which was purified by flash chromatography on silica using a 10:1 solution of ethyl acetate and triethylamine as eluant to give two products, both as colorless oils. The dihydrochloride salts of these two products were prepared by dissolution in ether and then saturation with gaseous hydrogen chloride. This gave 3-[1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)amino]-*N*-(2-pyrimidinyl)propylamine dihydrochloride **10** (0.80 g, 37%), mp 50−55 °C: ^{1}H NMR (DMSO-d_6) δ 9.91 (br s, 1H, HCl), 9.56 (br s, 1H, HCl), 8.96 (br s, 1H, NH), 8.61 (d, J = 5.2 Hz, 2H, pyrimidine 4,6-H), 6.87 (m, 5H, 4 aromatic H and pyrimidine 5-H), 4.74 (m, 1H), 4.42 (dd, J = 11.7, 2.3 Hz, 1H), 4.08 (dd, J = 11.7, 6.8 Hz, 1H), 3.57 (t, J = 6.5 Hz, 2H), 3.35 (m, 1H), 3.10 (m, 4H), and 2.04 (m, 2H). Anal. ($C_{16}H_{20}N_4O_2$·2HCl·0.1EtOAc, 0.7H_2O) C, H, Cl, N. The second product obtained was *N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-(2-pyrimidinyl)-3-(2-pyrimidinylamino)propylamine dihydrochloride **43** (0.46 g, 17%), mp 50−55 °C: ^{1}H NMR (DMSO-d_6) δ 8.99 (br s, 1H, HCl), 8.61 (d, J = 5.3 Hz, 2H, pyrimidine 4,6-H), 8.43 (d, J = 5.3 Hz, 2H, pyrimidine 4,6-H), 6.95 (t, J = 5.3 Hz, 1H, pyrimidine 5-H), 6.82 (m, 4 H, aromatic H), 6.73 (t, J = 5.3 Hz, 1H, pyrimidine 5-H), 6.30 (br s, 1H, NH), 4.55 (m, 1H), 4.35 (dd, J = 11.6, 2.2 Hz, 1H), 4.04 (dd, J = 11.6, 6.8 Hz, 1H), 3.70 (m, 6 H), and 1.95 (quintet, J = 6.4 Hz, 2H). Anal. ($C_{20}H_{22}N_6O_2$·2HCl, 0.2EtOAc, 1.4H_2O) C, H, Cl, N.

***(S)*-*N*-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-methyl-1-[1-(2-pyrimidinyl)-4-piperidyl]methylamine dihydrochloride (11).** A mixture of (*S*)-*N*-(2,3-dihydro-1,4-benzodioxan-2-ylmethyl)-1-[1-(2-pyrimidinyl)4-piperidyl]methylamine **1** (0.23 g, 0.56 mmol), sodium formate (0.13 g, 1.9 mmol), and formaldehyde (38−40% aqueous solution; 7 mL) was treated with formic acid (3 mL) while the temperature of the reaction was maintained between 5 and 10 °C. The mixture was then stirred at room temperature for 104 h, poured into water, and basified with 5 M aqueous sodium hydroxide solution. The cooled solution was then extracted with ether, washed with water, dried, and concentrated under reduced pressure to leave an oil (0.16 g). The oil was dissolved in ether and treated with hydrogen chloride gas giving (*S*)-*N*-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-methyl-1-[1-(2-pyrimidinyl)-4-piperidyl]methylamine dihydrochloride (**11**) (0.11 g, 46%) as a white solid, mp 235−240 °C: IR 3417 and 2800−2400 cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 10.68 (br s, 1H), 8.41 (d, J = 4.8 Hz, 2H, pyrimidine 4,6-H), 6.90 (m, 4H, aromatic H), 6.68 (t, J = 4.8 Hz, 1H, pyrimidine 5-H), 4.95 (m,1H), 4.65 (br d, J = 13.0 Hz, 2H), 4.36 (d, J = 11.4 Hz, 1H), 2.80−3.60 (m, 10H), 2.19 (m, 1H), 1.92 (m, 2H), and 1.16 (m, 2H), some peaks obscured by broad water peak δ 4.1. Anal. ($C_{20}H_{28}Cl_2N_4O_2$·1.2H_2O) C, H, N.

(−)-1-[1-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)piperid-4-yl]-*N*-(pyrimidin-2-yl)methylamine (8) and (+)-1-[1-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)piperid-4-yl]-*N*-(pyrimidin-2-yl)methylamine (9). A solution of 4-(aminomethyl)piperidine (11.63 g, 0.1 mol) and 5-chlorosalicylaldehyde (16.0 g, 0.1 mol) in a 1:1 mixture of ethanol and methanol (400 mL) was stirred at room temperature for 4.5 h. The solution was then concentrated under reduced pressure to give *N*-(5-chloro-2-hydroxybenzylidene)-1-piperid-4-ylmethylamine (**45**) (25.7 g, 100%) as a yellow solid which was used without purification.

A mixture of **45** (25.5 g, 0.1 mol) and the 4-toluenesulfonylate **25a** (16.97 g, 0.053 mol) in dry dimethyl sulfoxide (200 mL) was heated with stirring at 100 °C for 3 h. The cooled mixture was poured into water (400 mL) and extracted with ethyl acetate. The combined extracts were dried and evaporated under reduced pressure to afford a brown oil which solidified on standing. Recrystallization from ethanol furnished 1-[1-(1,4-benzodioxan-2-ylmethyl)piperid-4-yl]-*N*-(5-chloro-2-hydroxybenzylidene)methylamine (**46**) (14.02 g, 66%) as a yellow solid, mp 94−96 °C: ^{1}H NMR (DMSO-d_6) δ 13.71 (br s, 1H, OH), 8.52 (br s, 1H, N=CH), 7.55 (d, J = 2 Hz, 1H, aromatic H), 7.35 (dd, J = 8 Hz, 2 Hz, 1H, aromatic H), 6.85 (m, 5H, aromatic H), 4.30 (m, 2H) 3.93 (m, 1H), 3.50 (d, J = 5.5 Hz, 2H), 2.93 (m, 2H), 2.55 (m, 2H), 2.03 (br q, J = 12 Hz, 2H), 1.61 (m, 3H), and 1.26 (m, 2H).

A solution of the product from the previous reaction (14.0 g, 0.036 mol) in a 4:1 mixture of methanol and water (300 mL) and hydrochloric acid (5 M; 45 mL) was stirred at room temperature for 1.5 h. The mixture was then concentrated under reduced pressure, and the resulting mixture was washed with diethyl ether. The aqueous solution was then basified with 5 M sodium hydroxide and extracted with ethyl acetate. The combined extracts were dried and evaporated under reduced pressure to give 1-[1-(1,4-benzodioxan-2-ylmethyl)-piperid-4-yl]methylamine (8.00 g, 87%) as a brown oil: GC 96.6% (10% OV-7).

The amine product above (9.25 g, 0.035 mol) and 2-chloropyrimidine (4.01 g, 0.035 mol) were added to a mixture of toluene (300 mL) and triethylamine (14 mL), and the resulting

solution was stirred and heated at 95 °C for 72 h. The cooled mixture was evaporated, and the residue was dissolved in water (100 mL). The solution was extracted with ethyl acetate, and the combined extracts were dried and evaporated to leave a brown oil. Trituration with diethyl ether afforded a brown solid (1.55 g), mp 88−94 °C. The aqueous phase was re-extracted with ethyl acetate, the combined extracts were dried and evaporated, and the resulting brown oil was triturated with isopropyl alcohol to give additional brown solid (0.93 g), mp 94−96 °C. On standing, a solid precipitated from the filtrate and was collected by filtration. The solid was washed with diethyl ether to give a tan colored solid (1.01 g). The three solids isolated above were combined and washed with diethyl ether to give racemic 1-[1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)piperid-4-yl]-*N*-(pyrimidin-2-yl)methylamine (1.23 g, 10%) as a tan solid. The enantiomers were then separated by preparative chiral HPLC (column Chiralcel OD, mobile phase 20% ethanol in cyclohexane) to give (−)-1-[1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)piperid-4-yl]-*N*-(pyrimidin-2-yl)methylamine (0.44 g) as a solid, mp 88−90 °C: $[\alpha]_D = -24.6°$ (c = 1.0, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.23 (d, J = 5 Hz, 2H, pyrimidine 4,6-H), 7.17 (t, J = 6 Hz, 1H, HNCH$_2$), 6.81 (m, 4H, aromatic H), 6.51 (t, J = 5 Hz, 1H, pyrimidine 5-H), 4.29 (m, 2H), 3.93 (m, 1H), 3.15 (t, J = 6 Hz, 2H), 2.91 (m, 2H), 2.54 (m, 2H), 1.95 (br q, J = 12 Hz, 2H), 1.61 (m, 3H), and 1.20 (m, 2H), and (+)-1-[1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)piperid-4-yl]-*N*-(pyrimidin-2-yl)methylamine (0.52 g) as a solid, mp 84−87 °C; which was less enantiomerically pure as shown by its optical rotation $[\alpha]_D = +16°$ (c = 1.0, CH_2Cl_2): ^{1}H NMR (DMSO-d_6) δ 8.23 (d, J = 5 Hz, 2H, pyrimidine 4,6-H), 7.17 (t, J = 5 Hz, 1H, HNCH$_2$), 6.82 (m, 4H, aromatic H), 6.51 (t, J = 5 Hz, 1H, pyrimidine 5-H), 4.29 (m, 2H), 3.93 (m, 1H), 3.15 (t, J = 6 Hz, 2H), 2.88 (m, 2H), 2.52 (m, 2H), 1.97 (br q, J = 12 Hz, 2H), 1.62 (m, 3H), and 1.18 (m, 2H).

1-[1-(2,3-Dihydro-1,4-benzodioxin-2-ylmethyl)-4-piperidyl]-*N*-(2-methoxyphenyl)methylamine Oxalate (22). A stirred mixture of 2-(chloromethyl)-2,3-dihydro-1,4-benzodioxin[32] (20.0 g, 0.108 mol) and ethyl piperidine-4-carboxylate (34.0 g, 0.217 mol) was heated at 130 °C for 3 h. The mixture was then cooled, diluted with ether, and filtered. The filtrate was concentrated under reduced pressure, and volatile byproducts were removed by vacuum distillation. The residual oil was then purified by flash column chromatography on silica using a 30:1 solution of dichloromethane and industrial methylated spirit as eluant to give ethyl 1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)piperidine-4-carboxylate **37** (23.3 g, 71%) as a brown oil. Anal. ($C_{17}H_{23}NO_4$) C, H, N.

A solution of the ester **37** (5.0 g, 0.164 mol) in industrial methylated spirit (100 mL) was added to a stirred solution of potassium hydroxide (4.0 g, 0.071 mol) in water (50 mL), and the mixture was stirred at room temperature for 1 h and then at reflux for 6 h. The cooled solution was concentrated under reduced pressure, and the residue was diluted with water and neutralized with hydrochloric acid (5 M). The aqueous mixture was then concentrated under reduced pressure, and the residue was triturated with a 25:1 solution of dichloromethane and methanol. The inorganic material was removed by filtration, and the filtrate was concentrated under reduced pressure to give crude 1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)piperidine-4-carboxylic acid **38** (5.03 g).

Ethyl chloroformate (0.90 g, 8.30 mmol) was added dropwise to a stirred solution of the carboxylic acid **38** (2.52 g, 8.97 mmol) and triethylamine (1.08 g, 10.70 mmol) in chloroform (90 mL) at 0 °C. After 30 min a solution of 2-methoxyaniline (1.08 g, 8.80 mmol) in chloroform (45 mL) was added, and the mixture was stirred at room temperature overnight and then at 50 °C for 1 h. The cooled mixture was then concentrated under reduced pressure, and the residue was purified by flash column chromatography on silica using a 1:1 mixture of ethyl acetate and petroleum ether (bp 40−60 °C) as eluant to give 1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-*N*-(2-methoxyphenyl)piperidine-4-carboxamide **39** (1.05 g, 31%): ^{1}H NMR ($CDCl_3$) δ 8.39 (dd, J = 7.8, 1.7 Hz, 1H, aromatic H), 7.85 (br s, 1H, NH), 6.90 (m, 7H), 4.32 (m, 2H), 4.02 (m, 1H), 3.89 (s, 3H, OMe), 3.06 (m, 2H), 2.63 (m, 2H), 2.24 (m, 3H), and 1.90 (m, 4H).

A stirred solution of the amide **39** (1.69 g, 4.4 mmol) in dry tetrahydrofuran (100 mL) was treated with borane-dimethyl sulfide complex (1.0 M solution in tetrahydrofuran; 1.9 mL, 19 mmol), and the mixture was heated at reflux for 2 h. The mixture was cooled, and the solvent was removed under reduced pressure. Hydrochloric acid (2 M) was added to the residue, and the mixture was heated at 95 °C for 90 min. The mixture was cooled, basified with 5 M aqueous sodium hydroxide solution, and extracted with dichloromethane. The organic solution was washed with water, dried, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate, and a warm solution of oxalic acid (0.20 g, 16 mmol) in ethyl acetate was added. The resulting precipitated solid was collected by filtration and dried to give 1-[1-(2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-4-piperidyl]-*N*-(2-methoxyphenyl)methylamine oxalate **22** (0.78 g, 38%), mp 215−218 °C: IR 3424 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.80 (m, 6H, aromatic H), 6.51 (m, 2H, aromatic H), 4.63 (m, 1H), 4.29 (dd, J = 11.5, 2.3 Hz, 1H), 4.00 (m, 1H), 3.77 (s, 3H, OMe), 3.35 (m, 2H), 3.10 (m, 2H), 2.99 (d, J = 6.1 Hz, 2H), 2.73 (m, 2H), 1.83 (m, 3H), and 1.41 (m, 2H). Anal. ($C_{22}H_{28}N_2O_3(COOH)_2 \cdot 0.1H_2O$) C, H, N.

Binding Assays. Assay methods and radioligands for the cloned human dopamine D_{1-5} receptors and animal derived D_2, 5-HT$_{2A}$, α_1, α_{2D}, muscarinic, and H_1 receptors were as previously described.[3] For percent displacement of radioligand at 10^{-6} M the specific binding in the absence and presence of test compound was determined. The percentage displacement of specific binding was then calculated manually. For K_i determinations, the IC_{50} values were calculated using the iterative curve-fitting program EBDA. K_i values were then calculated using the Cheng and Prusoff equation.

5-HT$_{1A}$ Receptor Binding Assay in Rat Brain. 1. Membrane Preparation. Adult male CD rats were killed by cervical dislocation, their brains removed, and hippocampii (100−120 mg) immediately dissected. Tissue was homogenized in ice-cold 50 mM Tris-HCl, pH 7.7 (at 25 °C, 1:40 w/v), using a motor-driven Teflon pestle (12 strokes, 800 rpm) and centrifuged at 40000*g* for 10 min. To remove endogenous 5-HT, the pellet was rehomogenized in 50 mM Tris-HCl, pH 7.7 (1:40 w/v), incubated at 37 °C for 10 min, and then recentrifuged at 40000*g* for 10 min. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.7, containing 4 mM calcium chloride, 0.1% L-ascorbic acid, and 10 μM pargyline hydrochloride (equivalent to 6.25 mg wet weight of tissue/mL) and used immediately in the binding assay. All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (400 μL; equivalent to 2.5 mg wet weight of tissue/tube) were incubated with 50 μL of [^{3}H]8-OH-DPAT at a single concentration of 2 nM and 50 μL of distilled water (total binding) or 50 μL of test compound (10^{-6} M) or 50 μL of 5-HT (10 μM; nonspecific binding) for 30 min at 25 °C.

Membrane-bound radioactivity was recovered by filtration under vacuum through Skatron 11734 filters using a Skatron cell harvester. Filters were rapidly washed with ice-cold 50 mM Tris-HCl, pH 7.7 (wash setting 9,9,0), and radioactivity determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

5-HT$_{1B}$ Receptor Binding Assay in Pig Brain. 1. Membrane Preparation. Pig caudate was homogenized in ice-cold 50 mM Tris-HCl, pH 7.7 (at 25 °C, 1:100 w/v), using a motor-driven Teflon pestle (8 strokes, 800 rpm) and centrifuged at 40000*g* for 10 min. The resulting pellet was resuspended in 50 mM Tris-HCl, pH 7.7 (1:100 w/v), incubated at 37 °C for 10 min to remove endogenous 5-HT, and then recentrifuged at 40000*g* for 10 min. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.7 (equivalent to 33.3 mg wet weight of tissue/mL), and used immediately in the binding assay. All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (300 μL; equivalent to 10

mg wet weight of tissue/tube) were incubated with 50 μL of [^{3}H]sumatriptan at a single concentration of 4 nM; 50 μL of 50 mM Tris-HCl, pH 7.7; 50 μL of 50 mM Tris-HCl, pH 7.7, containing 50 mM calcium chloride, 100 μM pargyline hydrochloride, and 1% L-ascorbic acid; and 50 μL of distilled water (total binding), or 50 μL of drug solution (10^{-6} M), or 50 μL of 5-HT (10 μM; nonspecific binding) for 45 min at 25 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Whatman GF/B filters, previously soaked in 0.5% polyethylenimine using a Brandel cell harvester. Filters were rapidly washed with 16 mL of ice-cold 50 mM Tris-HCl, pH 7.7, and radioactivity was determined by liquid scintillation counting (2 mL Packard MV Gold scintillator).

5-HT$_{2C}$ Receptor Binding Assay in Pig Brain. 1. Membrane Preparation. Pig choroid plexus was homogenized in ice-cold 0.32 M sucrose (1:30 w/v) with a Kinematic polytron (speed setting 6 for 30 s) and centrifuged at 1000*g* for 10 min. The supernatant was stored on ice, and the pellet was rehomogenized in 0.32 M sucrose (1:15 w/v) and centrifuged at 850*g* for 12 min. Combined supernatants were diluted to 1:80 w/v with ice-cold 50 mM Tris-HCl, pH 7.4 (at 25 °C), and centrifuged at 40000*g* for 10 min. The resulting pellet was resuspended in 50 mM Tris-HCl, pH 7.4 (1:80 w/v), preincubated for 10 min at 37 °C to remove endogenous 5-HT, and centrifuged at 40000*g* for 10 min. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.4, containing 4 mM calcium chloride, 0.1% L-ascorbic acid, and 10 μM pargyline hydrochloride (equivalent to 12.5 mg wet weight of tissue/mL). All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (800 μL; equivalent to 10 mg wet weight of tissue/tube) were incubated with 100 μL of [^{3}H]mesulergine at a single concentration of 1 nM and 100 μL of distilled water (total binding) or 100 μL of drug solution (10^{-6} M or at 10 concentrations ranging from 10^{-11}−10^{-4} M) or 100 μL of 5-HT (10 μM; nonspecific binding) for 30 min at 37 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Whatman GF/C filters presoaked for 1 h in 0.5% polyethylenimine, using a Brandel cell harvester. Filters were rapidly washed with 12 mL of ice-cold 50 mM Tris-HCl, pH 7.4, and radioactivity determined by liquid scintillation counting (2 mL Packard MV Gold scintillator).

5-HT$_3$ Receptor Binding Assay in Rat Brain. 1. Membrane Preparation. Rat entorhinal cortices were homogenized in ice-cold 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (pH 7.4 at 4 °C; 1:10 w/v) using a Soniprep 150 (setting 5−6 for 8 s) and centrifuged at 49000*g* for 10 min. The resulting pellet was homogenized in 50 mM HEPES buffer (1:10 w/v) and recentrifuged at 49000*g* for 10 min. The final pellet was resuspended in 50 mM HEPES buffer, pH 7.4 (equivalent to 40 mg wet weight of tissue/mL), and used immediately in the binding assay. All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (200 μL; equivalent to 8 mg wet weight of tissue/tube) were incubated with 200 μL of [^{3}H]-GR 65630 at a single concentration of 0.2 nM and 100 μL of 50 mM HEPES buffer (total binding) or 100 μL of drug solution (10^{-6} M) or 100 μL of metoclopramide (30 μM; nonspecific binding) for 30 min at 37 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Skatron 11734 filters using a Skatron cell harvester. Filters were rapidly washed with ice-cold 50 mM HEPES buffer, pH 7.4 (wash setting 9,9,0), and radioactivity determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

5-HT$_4$ Receptor Binding Assay in Pig Brain. 1. Membrane Preparation. Pig hippocampii were homogenized in ice-cold 50 mM HEPES buffer (pH 7.4 at 4 °C; 1:15 w/v) using a Soniprep 150 (setting 5−6 for 8 s) and centrifuged at 40000*g* for 15 min. The resulting pellet was resuspended in 50 mM HEPES buffer, pH 7.4 (equivalent to 20 mg wet weight of tissue/mL), and used immediately in the binding assay. All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (400 μL; equivalent to 8 mg wet weight of tissue/tube) were incubated with 50 μL of [^{3}H]-GR 113808 at a single concentration of 0.1 nM and 50 μL of 50 mM HEPES buffer (total binding) or 50 μL of drug solution (10^{-6} M) or 50 μL of 5-HT (30 μM; nonspecific binding) for 30 min at 37 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Skatron 11734 filters, presoaked for 1 h in 0.5% polyethylenimine, using a Skatron cell harvester. Filters were rapidly washed with ice-cold 50 mM HEPES buffer, pH 7.4 (wash setting 9,9,0), and radioactivity determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

Rat Recombinant 5-HT$_6$ Receptor Binding Assay. 1. Membrane Preparation. Frozen membranes from SF9 insect cells, infected with baculovirus to express the rat recombinant 5-HT$_6$ receptor, were used. Membrane fragments were thawed, resuspended in 50 mM Tris-HCl, pH 7.4 (at 25 °C), containing 10 mM magnesium sulfate and 0.5 mM ethylenediaminetetraacetic acid (EDTA) (equivalent to 35 μg of membrane protein/mL), and used immediately in the binding assay.

2. Binding Assay. Membranes (400 μL; equivalent to 14 μg of membrane protein/tube) were incubated with 50 μL of [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) at a single concentration of 3 nM and 50 μL of distilled water (total binding) or 50 μL of drug solution (10^{-6} M) or 50 μL of methiothepin (10 μM; nonspecific binding) for 90 min at 27 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Whatman GF/C filters, presoaked for 1 h in 0.3% polyethylenimine, using a Skatron cell harvester. Filters were washed with ice-cold 50 mM Tris-HCl, pH 7.4 (wash setting 9,9,0), and radioactivity was determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

Rat Recombinant 5-HT$_7$ Receptor Binding Assay. 1. Membrane Preparation. Frozen membranes from SF9 insect cells, infected with baculovirus to express the rat recombinant 5-HT$_7$ receptor, were used. Membrane fragments were thawed, resuspended in 50 mM Tris-HCl, pH 7.4 (at 25 °C), containing 10 mM magnesium sulfate and 0.5 mM EDTA (equivalent to 5 μg of membrane protein/mL), and used immediately in the binding assay.

2. Binding Assay. Membranes (400 μL; equivalent to 2 μg of membrane protein/tube) were incubated with 50 μL of [^{3}H]-lysergic acid diethylamide ([^{3}H]LSD) at a single concentration of 3 nM and 50 μL of distilled water (total binding) or 50 μL of drug solution (10^{-6}M) or 50 μL of methiothepin (10 μM; nonspecific binding) for 90 min at 27 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Whatman GF/C filters, presoaked for 1 h in 0.3% polyethylenimine, using a Skatron cell harvester. Filters were washed with ice-cold 50 mM Tris-HCl, pH 7.4 (wash setting 9,9,0), and radioactivity determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

α_1 Adrenoceptor Binding in Postmortem Human Brain. 1. Membrane Preparation. Postmortem human cortex was homogenized in ice-cold 0.25 M sucrose (1:30 w/v) using a motor-driven Teflon pestle and centrifuged at 1000*g* for 10 min. The supernatant was stored on ice, and the pellet was resuspended in 0.25 M sucrose (1:15 w/v) and centrifuged at 750*g* for 10 min. Combined supernatants were diluted to 1:80 w/v with ice-cold 50 mM Tris-HCl, pH 7.4 (at 25 °C), and centrifuged at 28000*g* for 10 min. The resulting pellet was stored at −80 °C until the day of assay. The pellet was then resuspended in 50 mM Tris-HCl, pH 7.6 (1:40 w/v), and centrifuged at 40000*g* for 10 min. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.6 (equivalent to 12.5 mg wet weight of tissue/mL), and used immediately in the binding assay. All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (400 μL; equivalent to 5 mg wet weight of tissue/tube) were incubated with 50 μL of [^{3}H]-prazosin at a single concentration of 0.1 nM and 50 μL of distilled water (total binding) or 50 μL of drug solution (10^{-6} M or a range of 10 concentrations) or 50 μL of phentolamine (5 μM; nonspecific binding) for 30 min at 30 °C. Membrane-bound radioactivity was recovered by filtration under vacuum

through Skatron 11734 filters using a Skatron cell harvester. Filters were rapidly washed with ice-cold 50 mM Tris-HCl, pH 7.6 (wash setting 9,9,0), and radioactivity determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

5-HT_{1A} Receptor Binding in Postmortem Human Brain. 1. Membrane Preparation. Postmortem human hippocampi were homogenized in ice-cold 0.25 M sucrose (1:30 w/v) using a motor-driven Teflon pestle and centrifuged at 1000*g* for 10 min. The supernatant was stored on ice and the pellet was resuspended in 0.25 M sucrose (1:15 w/v) and centrifuged at 850*g* for 10 min. Combined supernatants were diluted to 1:80 w/v with ice-cold 50 mM Tris-HCl, pH 7.7 (at 25 °C), and centrifuged at 40000*g* for 10 min. The pellet was then resuspended in 50 mM Tris-HCl, pH 7.7 (1:40 w/v), and preincubated at 37 °C for 10 min to remove endogenous 5-HT. The membrane suspension was then centrifuged at 40000*g* for 10 min. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.7, containing 4 mM calcium chloride, 0.1% L-ascorbic acid, and 10 μM pargyline hydrochloride (equivalent to 12.5 mg wet weight of tissue/mL) and used immediately in the binding assay. All centrifugations were carried out at 4 °C.

2. Binding Assay. Membranes (400 μL; equivalent to 5 mg wet weight of tissue/tube) were incubated with 50 μL of [^{3}H]8-OH-DPAT at a single concentration of 0.75 nM and 50 μL of distilled water (total binding) or 50 μL of drug solution (10^{-6} M or a range of 10 concentrations) or 50 μL of 5-HT (10 μM; nonspecific binding) for 30 min at 25 °C. Membrane-bound radioactivity was recovered by filtration under vacuum through Skatron 11734 filters using a Skatron cell harvester. Filters were rapidly washed with ice-cold 50 mM Tris-HCl, pH 7.7 (wash setting 9,9,0), and radioactivity determined by liquid scintillation counting (1 mL Packard MV Gold scintillator).

Biochemical Assays. Levels of the 5-HT precursor 5-HTP in rat brain were determined according to the method of Heal et al.[33] Levels of the dopamine metabolites DOPAC and HVA were determined by a modification of the method of Digory and Buckett.[34]

Behavioral Tests. 1. Antagonism of Apomorphine-Induced Climbing. Groups of 10 male CD1 mice (20−25 g; Charles River, U.K.) received appropriate concentrations of test compound or vehicle (0.1 mL/kg) po, at appropriate times prior to 0.88 mg/kg apomorphine sc. Immediately after the dose of apomorphine, mice were placed individually into inverted, open-ended wire cages and climbing behavior was assessed on a simple 0−2 ranking scale, at 10 and 20 min after apomorphine administration. ED_{50} values (doses causing 50% of control score) and 95% confidence limits were calculated by an adaption of the method of Litchfield and Wilcoxon.[35]

2. Antagonism of Amphetamine-Induced Hyperlocomotion. Male CD rats (150−240 g; Charles River, U.K.) were placed individually into plexiglass test cages and allowed to acclimatize fully prior to testing. Groups of 8 rats received test compound or vehicle (1 mL/kg) ip, at appropriate times prior to 2.5 mg/kg *d*-amphetamine sulfate, sc. Locomotor activity was assessed by infrared detectors for 15 min following the administration of amphetamine, and ED_{50} values (doses causing 50% of control activity counts) and 95% confidence limits were calculated by an adaption of the method of Litchfield and Wilcoxon.[35]

3. Catalepsy. Groups of five male rats (130−190 g; Charles River, U.K.) were injected ip with test compound solution (1 mL/kg) at appropriate concentrations. Catalepsy was assessed at three time points, by gently placing each rat paw on a 45 mm rubber bung, in turn. A score of 1 was given for every paw that remained on the bung for 15 s (maximum score for group: 20 = 100% catalepsy). ED_{50} values were determined from the percent catalepsy at the final reading. ED_{50} determination was by an adaption of the method of Litchfield and Wilcoxon.[35]

Determination of 5-HT_{1A} receptor agonism by use of the Porsolt test, in the presence and absence of 8-OH-DPAT, was carried out using the method of Luscombe et al.[36]

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