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N,N'-DISUBSTITUTED ACETAMIDINES AS HIGH AFFINITY SIGMA RECEPTOR LIGANDS

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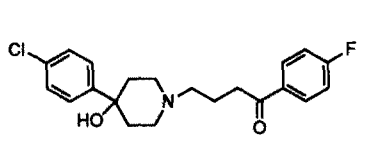
Abstract. A series of *N,N'*-disubstituted acetamidines was prepared as mammalian brain sigma receptor ligands. The *in vitro* binding affinities of seven representative ligands were measured using guinea pig brain membrane suspensions. *N*-1-adamantyl-*N'*-*o*-tolyl acetamidine is the most potent ligand in the series (IC_{50} = 6 nM vs [^{3}H]-DTG). All ligands showed low affinity toward the PCP site.

The pharmacological properties of many classical antipsychotic drugs such as haloperidol (**1**) and perphenazine (**2**) include a high affinity for the sigma receptor[1] suggesting that the sigma receptor may play an important role in mediating their antipsychotic activity.[2] Several atypical antipsychotic agents including BMY 14802 (**3**) have also been shown to bind tightly to the sigma receptor.[3] In animal models as well as in clinical trials, the atypical antipsychotics are devoid of severe extrapyramidal side effects typically associated with dopamine D_2 receptor antagonists.[4] Collectively these results suggest that sigma receptor ligands may be effective antipsychotic drugs without having extrapyramidal side effects in the treatment of schizophrenia.



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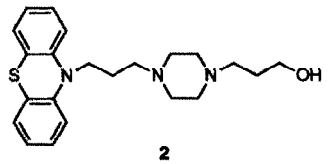

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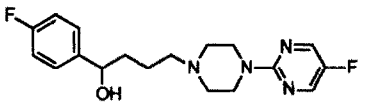

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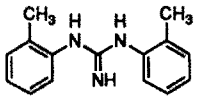

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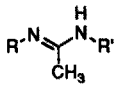

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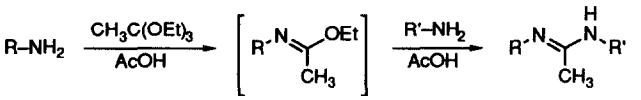
Earlier we described a series of *N,N'*-disubstituted guanidines based on the lead compound *N,N'*-di-*o*-tolyl guanidine (DTG, **4**) showing high affinity and selectivity for the sigma receptor.[5] Amidines share some of the structural and electronic characteristics of the guanidines including high basicity owing to resonance stabilization in the cationic form and the possibility of substitution on both nitrogen atoms. Herein we report the synthesis and pharmacological evaluation of a series of

N,N'-disubstituted acetamidines **5** with structures patterned after several of the best sigma receptor ligands discovered in the *N,N'*-disubstituted guanidine series.[5]

Chemistry. The acetamidines were prepared by adaptation of the procedure of Taylor and Ehrhart.[6] The synthesis of symmetrical acetamidines is illustrated by the synthesis of *N,N'*-di-*o*-tolyl acetamidine (**9**). A mixture of triethyl orthoacetate (3.24 g, 0.020 mol), *o*-toluidine (4.28 g, 0.040 mol) and glacial acetic acid (1 mL) was heated under reflux. The temperature was raised to 140 °C over 2 h and volatile material was removed under vacuum (20 mm). The residual syrup was taken up in ether and treated with sodium bicarbonate solution. Evaporation of the ether layer followed by recrystallization from hexane-5% toluene gave 1.85 g (39%) of **9** as colorless crystals, mp 65-68 °C (lit.[6] mp 69-70.5 °C). After chromatographic purification, the free base was converted to the hydrochloride salt, mp 237-239 °C.

The synthesis of unsymmetrical amidines is illustrated by the synthesis of *N*-1-adamantyl-*N'*-*o*-tolyl acetamidine (**6**). A mixture of 1-adamantanamine (50 g, 0.33 mol), triethyl orthoacetate (221 g, 1.36 mol) and acetic acid (4 mL) was heated at reflux for 6 h. Volatiles were distilled off over the next 18 h and then the mixture was concentrated to dryness at 55 °C (12 mm) to afford 72 g (99%) of ethyl *N*-1-adamantanylacetimidate as a creamy white solid, mp 62-64 °C. This sample and *o*-toluidine (34.5 g, 0.322 mol) was heated at 110 °C to a melt, then acetic acid (5 mL) was added. After heating at 120 °C for 5 h, the mixture was cooled to 25 °C and treated with 0.7 M HCl-MeOH to give a solid which was crystallized from 95% ethanol giving 27.9 g (27%) of **6**•HCl as white crystals, mp 281-282 °C.

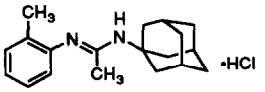
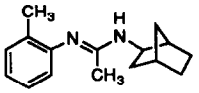
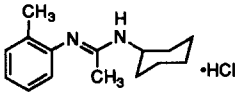
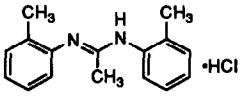
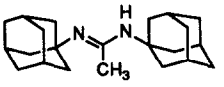
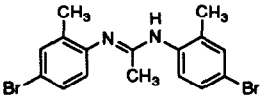
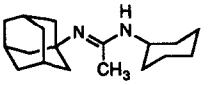


Receptor-Ligand Binding Assays. [^{3}H]-DTG binding to sigma receptors was assayed in guinea pig membranes as described in Weber et al.[8] Nonspecific binding was determined using 10 μM haloperidol. NMDA receptor ion-channel site binding experiments were performed using [^{3}H]-MK 801 and rat brain membranes as described in Keana et al.[9] Nonspecific binding was determined using 10 μM PCP. Rat brain membranes were incubated with 0.01% Triton X-100 then washed three times by centrifugation to reduce the endogenous amino acid concentrations.

Results and Discussion

Three symmetrical acetamidines **9-11** and four unsymmetrical acetamidines **6-8** and **12** (Table I) were synthesized and evaluated in *in vitro* displacement experiments with guinea pig membrane homogenate using the sigma receptor ligand [^{3}H]-DTG. Since certain sigma receptor ligands exhibit cross-reactivity with the NMDA receptor ion-channel site (PCP receptor),[7] the binding affinity of the acetamidines toward the NMDA receptor ion-channel site was determined using rat brain membrane homogenate and the NMDA receptor ion-channel site specific ligand [^{3}H]-MK 801 [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-10-imine].

Table I. Binding Affinities of *N,N'*-Disubstituted Acetamidines for the Sigma Receptor Site and the NMDA Receptor Ion-Channel Site

Compd[10]	Structure	mp °C	Sigma (nM) IC_{50} vs ^{3}H-DTG			NMDA ion-channel site (nM) IC_{50} vs ^{3}H-MK 801		
			mean	sem	n	mean	sem	n
6		281-282	6	2	6	10,000	—	2
7		149-151	9	0.2	3	80,000	—	1
8		215-217	9	2	5	10,000	—	2
9		237-239	15	1	4	10,000	—	2
10		235-236	16	2	6	10,000	—	4
11		199-200.5	26	3	4	9,112	800	2
12		265-268	72	12	4	10,000	—	2

The first compound tested in the symmetrical series was **9**. This ligand showed a higher affinity (IC_{50} = 15 nM vs ^{3}H-DTG) for the sigma receptor than its guanidine counterpart, *N,N'*-di-*o*-tolyl guanidine (**4**; IC_{50} = 32 nM). Similarly *N,N'*-di-1-adamantyl acetamidine (**10**) was found to be equipotent to its guanidine counterpart (16 nM vs 17.5 nM). Compound **11**, a dibromo analog of **9** was prepared as a precursor for introducing tritium into **9** for possible use as a radioligand.

The unsymmetrical acetamidine **6** showed a threefold greater affinity for the sigma receptor compared to the symmetrical counterparts **9** or **10**. The 1-adamantyl substituent of compound **6** was replaced with a norbornyl group (**7**) and cyclohexyl group (**8**). Both of these amidines showed high affinity and selectivity for the sigma receptors. When the *o*-tolyl substituent of compound **6** was replaced with a cyclohexyl to give compound **12**, affinity towards sigma receptor is reduced about

twelvefold. This indicates that the *o*-tolyl group is a preferred substituent for high activity at the sigma receptor.

In summary, the effect of substituent changes on sigma receptor affinity in the amidine series was comparable to that observed with the corresponding guanidines. The approximately parallel affinity of the two series indicates that the unsubstituted nitrogen atom in the guanidines is not absolutely required for sigma receptor activity and may be replaced by a methyl group without substantially affecting the affinity for the sigma receptor.

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References and Notes

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