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Synthesis of Clozapine Analogues and Their Affinity for Clozapine and Spiroperidol Binding Sites in Rat Brain[1]

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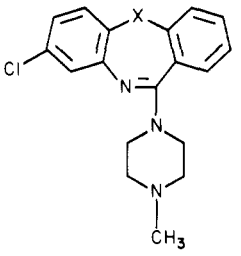
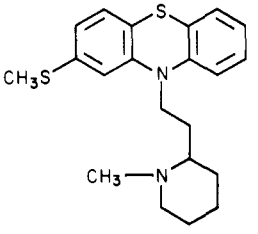
Analogues of clozapine, some prepared by a novel, shorter synthesis than those described previously, were evaluated as potential antipsychotic agents using clozapine binding sites in rat forebrain that are nonmuscarinic and nondopaminergic in nature and from which [^{3}H]clozapine is displaced by known antipsychotic agents. The binding of clozapine to muscarinic sites is inhibited in the presence of atropine. Displacement of [^{3}H]clozapine by an analogue of clozapine in the presence of atropine represents nonmuscarinic binding, while displacement in the absence of atropine represents muscarinic (cholinergic) plus nonmuscarinic binding. The relative affinity of the analogues for dopamine binding sites was determined by their ability to displace [^{3}H]spiroperidol from binding sites in rat caudate nuclei. To the extent which binding affinity for nonmuscarinic clozapine sites in rat forebrain reflects the antipsychotic potential of a particular drug, dibenzo-5*H*-cycloheptene analogues of clozapine are as effective as clozapine itself. Strong binding to nonmuscarinic clozapine sites is not dependent on the presence of a clorine atom on the tricyclic system. One or both of the nitrogen atoms in the dibenzo-5*H*-[1,4]diazepine ring of clozapine appear to be necessary for the strong inhibition of clozapine binding to spiroperidol sites in rat caudate nuclei. Anticholinergic activity is substantially higher for clozapine and its dibenz[1,4]oxazepine analogue than for its benzo-5*H*-cycloheptene analogue.

Neuroleptic-induced extrapyramidal disorders limit the usefulness of most currently available antipsychotic drugs.[2] The extrapyramidal side effects, however, are not necessarily an essential part of antipsychotic efficacy.[3] Drugs like clozapine [8-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4]diazepine](**1a**) and, to a lesser extent,





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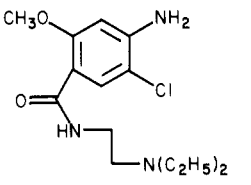
1a, X = NH
b, X = NCH_3
c, X = O

thioridazine (**2**) are virtually free of extrapyramidal side effects while having good antipsychotic activity. Conversely, there are drugs like metoclopramide (**3**) which display a neuroleptic profile (extrapyramidal disturbances) associated with antidopaminergic activity, similar to that produced by phenothiazines and butyrophenones,[4,5] but



3

which appear to have weak antipsychotic activity.[6] Thus, pharmacological separation of extrapyramidal from antipsychotic activity seems clearly feasible.

Since clozapine (**1a**) has displayed agranulocytosis as a serious side effect,[7] we have reinvestigated the pharmacology of some analogues of clozapine (**1a**) in the pursuit of an antipsychotic agent free of extrapyramidal and possibly other toxic effects. On this basis, we have considered analogues of **1a** which might reveal the unique, minimum structural requirements for antipsychotic activity and at the same time be relatively free of any serious side effects.

For these analogues, emphasis was placed on alteration and replacement of the nitrogen atoms in **1a**. It has already been shown that the bridge secondary nitrogen atom in **1a** can be replaced with other atoms, such as sulfur or oxygen, and still produce strong central depressant activity.[8,9] To this end, we have evaluated, in comparison with

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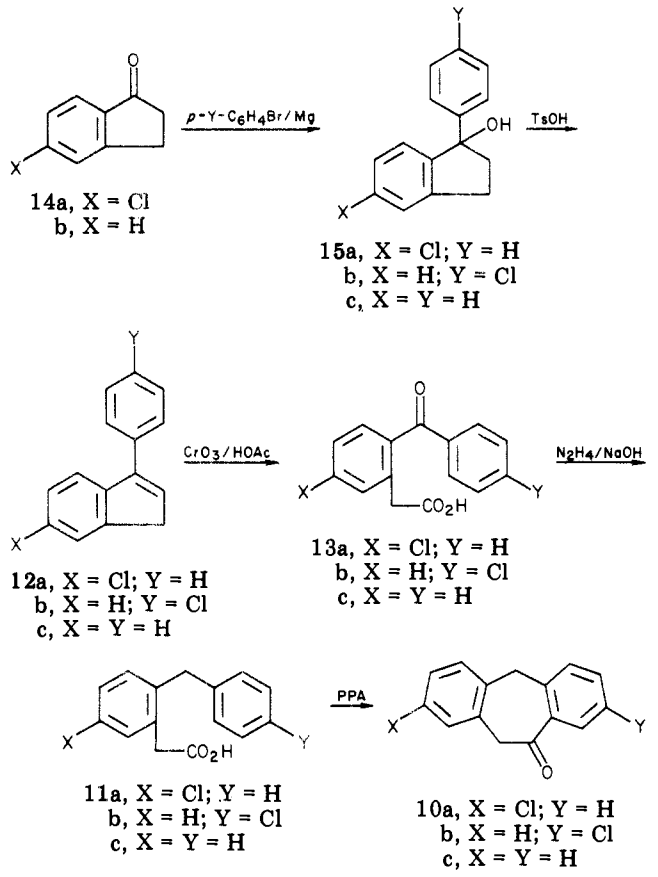
Scheme I



14a, X = Cl
b, X = H

15a, X = Cl; Y = H
b, X = H; Y = Cl
c, X = Y = H

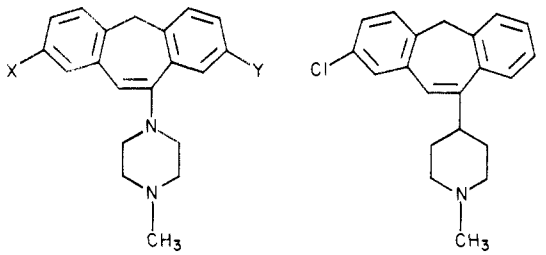
12a, X = Cl; Y = H
b, X = H; Y = Cl
c, X = Y = H

13a, X = Cl; Y = H
b, X = H; Y = Cl
c, X = Y = H

11a, X = Cl; Y = H
b, X = H; Y = Cl
c, X = Y = H

10a, X = Cl; Y = H
b, X = H; Y = Cl
c, X = Y = H

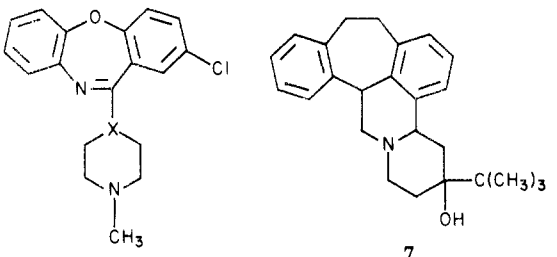
1a, *N*-methylclozapine[10] (**1b**), 8-chloro-11-(4-methylpiperazino)dibenz[*b,f*][1,4]oxazepine[11] (**1c**), 2-chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]cycloheptene[12] (**4a**),



4a, X = Cl; Y = H
b, X = H; Y = Cl
c, X = Y = H

5

and 2-chloro-10-(1-methyl-4-piperidyl)-5*H*-dibenzo[*a,d*]-cycloheptene[13] (**5**). The analogues **1b** and **1c** were secured from outside sources, while **4a** and **5** were prepared by a novel, shorter synthesis than those described previously[12,13] in connection with the search for substances with neuroleptic activity without recognition of their potential as nonneuroleptic antipsychotic agents.[13] Since one of our objectives is to find the structural features of clozapine (**1a**) which effect its binding to dopamine receptors, interest in **5** was increased by the recent report[14] that the 1-methyl-4-piperidyl analogue (**6a**) of loxapine (**6b**) is ca. 300 times less potent in the antiapomorphine assay than is **6b**.

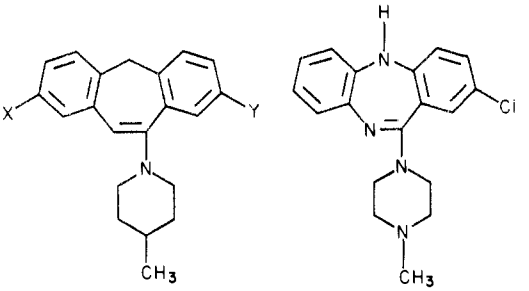


6a, X = CH
b, X = N

7

The importance of the nitrogen atoms in the piperazino moiety in clozapine (**1a**) was further examined since it has been suggested that the antipsychotic activity of **1a** is due to its structural resemblance to butaclamol (**7**),[15] the nitrogen atom at the 1-piperazinyl position in **1a** corresponding to the tertiary amino group in **7**. Thus, another analogue of **1a**, 2-chloro-10-(4-methylpiperidino)-5*H*-dibenzo[*a,d*]cycloheptene (**8a**), was prepared and evaluated.

The effect of the chloro substituent in clozapine (**1a**) was also assessed, since 2-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine[10] (**9**) has been reported as a



8a, X = Cl; Y = H
b, X = H; Y = Cl

9

typical potent neuroleptic.[9] Thus, **9** was compared to **1a**, and 2-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]-cycloheptene[12] (**4b**), 2-chloro-11-(4-methylpiperidino)-5*H*-dibenzo[*a,d*]cycloheptene (**8b**), and 10-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]cycloheptene[16] (**4c**) were also prepared and evaluated.

The problem of finding a drug with a clozapine-like profile using the classical neuroleptic testing methods is well known.[4] Recently it has been demonstrated that clozapine (**1a**) binds to nonmuscarinic sites in rat forebrain that appear not to be dopaminergic.[17] While the exact nature of these nonmuscarinic sites and their relationship to the antipsychotic properties of clozapine (**1a**) are unknown, displacement of [^{3}H]clozapine from these sites by antipsychotic drugs[17,18] suggested the use of these sites as an assay method for the evaluation of the analogues of **1a** as potential antispsychotic agents. Thus, the analogues of **1a** were compared to **1a** in their respective abilities to inhibit the binding of [^{3}H]clozapine. The inhibition of

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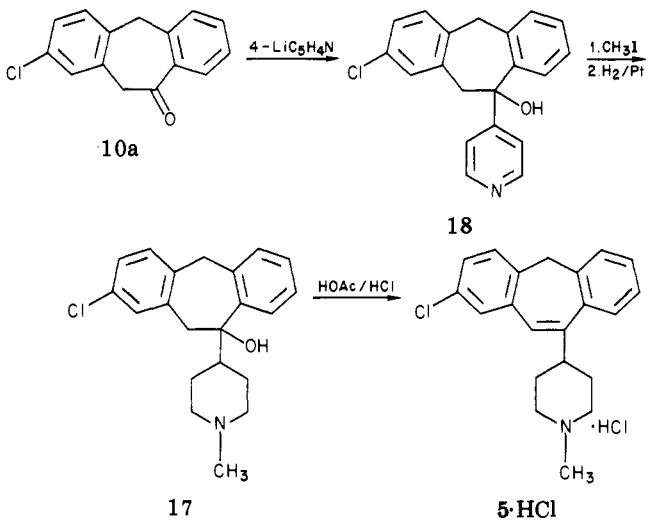
Table I. Inhibition of Muscarinic and Nonmuscarinic [^{3}H]Clozapine and [^{3}H]Spiroperidol Binding in Rat Brain in Vitro

	IC_{50} ± SEM,[a] nM		
	[^{3}H]clozapine binding[b]		
no.	muscarinic	nonmuscarinic[c]	[^{3}H]spiroperidol binding[d]
1a	12 ± 2 (17)	13 ± 2 (19)	4310 ± 226 (3)
1b	12 ± 3 (6)	88 ± 27 (6)	32 400 ± 1510 (3)
1c	49 ± 9 (6)	38 ± 13 (6)	2480 ± 100 (3)
4a	670 ± 170 (6)	13 ± 3 (6)	342 ± 16 (3)
4b	290 ± 98 (6)	10 ± 5 (6)	39 ± 4 (3)
4c	610 ± 120 (5)	3.7 ± 0.4 (5)	808 ± 222 (4)
5	622 ± 74 (6)	7 ± 2 (6)	678 ± 38 (3)
8a	>10 000	>10 000	>10 000
8b	>10 000	>10 000	e
9	e	17 ± 4 (4)	758 ± 167 (3)

[a] An IC_{50} value designates the concentration of clozapine (1a) or of a clozapine analogue that displaces specific binding of the radiolabeled ligand by 50%. SEM is the standard error of the mean for the number of displacement curves, given in parentheses, from which the IC_{50} value was calculated. [b] [^{3}H]Clozapine concentration was 2.1 nM. [c] Binding in the presence of 1 μM atropine. [d] [^{3}H]Spiroperidol concentration was 2.2 nM. [e] Not determined.

Scheme II



10a 18 17 5·HCl

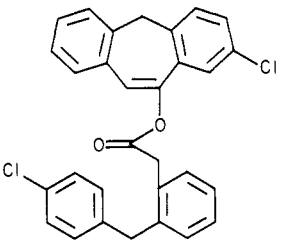
[^{3}H]clozapine binding in the presence of atropine represents nonmuscarinic binding,[17] while binding in the absence of atropine represents muscarinic plus nonmuscarinic binding. Thus, the binding of **1a** and the analogues of **1a** with cholinergic receptor sites was determined by the difference between [^{3}H]clozapine binding in the absence and presence of atropine. The relative affinity for dopamine receptor sites by these compounds was determined by their ability to displace [^{3}H]spiroperidol from binding sites in tissue from rat caudate nuclei.[19]

Results and Discussion

Syntheses. For synthesis of the seven-membered carbocyclic analogues of clozapine, the key intermediates are the tricyclic ketones **10a–c**, prepared by cyclization of the corresponding 2-benzylphenylacetic acids **11a–c** in polyphosphoric acid[20] (PPA) (Scheme I).

The previously reported routes[20,21] to the acids **11a–c** involve extensive multistep syntheses from starting materials not readily available. A more efficient route to **11a–c** employed here and used earlier for the preparation of **11c**[22] involves the chromic acid oxidation of the double bond in the 1-phenylindenes **12a–c**, followed by Wolff–Kishner reduction of the resulting 2-benzoylphenylacetic acids **13a–c**. Thus, 5-chloro-1-indanone[23] (**14a**) was prepared by cyclization of *m*-chlorohydrocinnamic acid.[24] A Grignard reaction with bromobenzene or *p*-bromochlorobenzene on the appropriate 1-indanone **14a** or **14b** gave the carbinols **15a–c**. Dehydration with *p*-toluenesulfonic acid (TsOH) gave the corresponding indenes **12a–c**.

In the cyclization of **11b** to form **10b**, a considerable amount of the enol ester **16** was formed, especially when the temperature of the reaction was allowed to rise to 135 °C. The structure of **16** was deduced from its proton nuclear magnetic resonance spectrum and its hydrolysis to **10b** and **11b**.



16

All of the enamines **4a–c** and **8a,b** were synthesized in one step by condensation of *N*-methylpiperazine or 4-methylpiperidine with the apropriate tricyclic ketone using titanium tetrachloride as catalyst and water scavenger.[25] This latter reaction is more direct than the earlier reported[12,16] multistep introduction of the heterocyclic group by conversion of the tricyclic ketones to the corresponding 10-bromo-5*H*-dibenzo[*a*,*d*]cycloheptenes.

An attempted preparation of 2-chloro-10,11-dihydro-10-(1-methyl-4-piperidyl)-5*H*-dibenzo[*a*,*d*]cyclohepten-10-ol (**17**) by the addition of 1-methyl-4-piperidylmagnesium chloride to the carbonyl group in **10a** was not successful, possibly because of the strong tendency of the tricyclic ketone to form its enolate salt. The hydroxy compound **17** was prepared (Scheme II) by the addition of 4-pyridyllithium to **10a**[13] and subsequent methylation and catalytic reduction of **18**. Dehydration of **17** in acetic acid–hydrochloric acid gave **5** as the hydrochloride salt.

Biological Activity. As seen in Table I, replacement of both nitrogen atoms with carbon atoms in the seven-membered ring of clozapine (**1a** vs. **4a**) does not appre-

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ciably affect affinity for nonmuscarinic clozapine binding sites. In contrast, this increases binding affinity at dopamine sites and decreases affinity at muscarinic sites. Replacement of the bridge nitrogen atom with oxygen (**1a** vs. **1c**) results in slightly reduced affinity at both muscarinic and nonmuscarinic clozapine binding sites but increases binding affinity at dopamine sites. The addition of a methyl group to the bridge nitrogen atom (**1a** vs. **1b**) does not affect muscarinic properties but decreases affinity to both dopamine and nonmuscarinic clozapine sites.

The proximity of the chlorine atom to the piperazino group does not appear to affect binding affinity at nonmuscarinic clozapine binding sites (**1a** vs. **9**; **4a** vs. **4b**). However, this relationship is important at dopamine binding sites, since both **9** and **4b** are more potent at displacing [^{3}H]spiroperidol than the corresponding analogues with the chlorine atom in a distal position (**1a** and **4a**). Removal of the chlorine atom (**4c**) causes a slight decrease in affinity at dopamine sites and a slight increase in affinity at nonmuscarinic clozapine sites as compared to the two chlorinated derivatives (**4a** and **4b**).

Replacement of the nitrogen atom at the 1-piperazinyl position of **4a** by a carbon atom (**5**) has little effect on binding affinity at dopamine, muscarinic or nonmuscarinic clozapine sites. In contrast, replacement of the nitrogen atom at the 4-piperazinyl position by a carbon atom (**8a** and **8b**) drastically reduces the affinity at all three sites.

Conclusions

As far as the binding affinity at nonmuscarinic clozapine sites in rat forebrain reflects the antipsychotic potential of a particular drug, the dibenzo-5*H*-cycloheptene analogues **4a–c** and **5** of clozapine (**1a**) are as effective as **1a** itself. Strong binding to nonmuscarinic sites is not dependent on the presence of a chlorine atom on the tricyclic system or of a nitrogen atom at the attachment bond of the six-membered heterocyclic group.

Regardless of the presence or position of a chlorine atom, the dibenzo-5*H*-cycloheptene analogues **4a–c** are substantially more potent than clozapine (**1a**) in the spiroperidol binding assay, but the increase in dopaminergic activity on shifting the chlorine atom in relation to the piperazino group from the distal to the proximal position is similar, six times for the clozapine isomers (**1a** and **9**) and nine times for the carbocyclic isomers (**4a** and **4b**). One or both of the nitrogen atoms in the dibenzo-5*H*-[1,4]diazepine ring of **1a** appear to be necessary for the strong inhibition of clozapine binding to spiroperidol sites.

Anticholinergic activity, as measured by binding to muscarinic sites, is substantially higher for clozapine (**1a**) and its dibenz[1,4]oxazepine analogue (**1c**) than for its dibenzo-5*H*-cycloheptene analogue (**4a**).

Experimental Section

Melting points were taken in open capillary tubes and are corrected. All compounds designated as pure showed a single spot on thin-layer chromatography on silica gel using an appropriate eluting solvent and had proton magnetic resonance spectra compatible with their assigned structures. These spectra were obtained in chloroform-*d* (**5** used in place of **5**·HCl), using a JEOL JNM-MH-100 spectrometer with tetramethylsilane as an internal standard. Microanalyses were done by Galbraith Laboratories, Knoxville, TN, and agreed to within 0.4% of the calculated values unless otherwise noted.

***N*-Methylclozapine (1b) and 8-chloro-11-(4-methylpiperazino)dibenz[*b,f*][1,4]oxazepine** (**1c**) were gifts from Dr. Jean Schmutz, Wander Ltd., Berne, and were light yellow solids with mp 164–165 °C (lit.[10] mp 164–165 °C) and 164–165 °C (lit.[11] mp 165–166 °C), respectively.

2-Chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]cycloheptene (**4a**). A solution of $TiCl_4$ (1.1 g, 5.8 mmol) in benzene (35 mL) was slowly added under nitrogen to a stirred solution of 2-chloro-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-10-one (**10a**; 2.3 g, 9.5 mmol) and *N*-methylpiperazine (4.4 g, 44 mmol) in benzene (35 mL) at 20 °C, and the mixture was boiled for 2 h. After cooling, the solution was poured into a mixture of $NaHCO_3$ (2.0 g, 24 mmol) in water (200 mL). The benzene layer was separated, and the TiO_2 which was suspended between the benzene and aqueous phases was discarded. The aqueous phase was extracted with ether (3 × 100 mL), and the ether was added to the benzene solution. This mixture was dried (Na_2SO_4) and evaporated. Crystallization of the residue with isopropyl ether and recrystallization from the same solvent gave pure **4a** (1.3 g, 42%), mp 151–153 °C (lit.[12] mp 152 °C). Anal. ($C_{20}H_{21}ClN_2$) C, H, N.

2-Chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]cycloheptene (**4b**) was prepared from **10b** as described for the preparation of **4a** from **10a**. Recrystallization from isopropyl ether gave pure **4b** (85%), mp 134–135 °C (lit.[12] mp 133 °C). Anal. ($C_{20}H_{21}ClN_2$) C, H, N.

10-(4-Methylpiperazino)-5*H*-dibenzo[*a,d*]cycloheptene (**4c**) was prepared from **10c** as described for the preparation of **4a** from **10a**. Recrystallization from isopropyl ether gave pure **4c** (42%), mp 134–135 °C (lit.[16] mp 133–135 °C). Anal. ($C_{20}H_{22}N_2$) C, H, N.

2-Chloro-10-(1-methyl-4-piperidyl)-5*H*-dibenzo[*a,d*]cycloheptene Hydrochloride (**5**·HCl). A mixture of 2-chloro-10,11-dihydro-10-(4-pyridyl)-5*H*-dibenzo[*a,d*]cycloheptene-10-ol (**18**; 0.56 g, 1.7 mmol) and methyl iodide (0.28 g, 2.0 mmol) in acetonitrile (10 mL) was boiled for 17 h. Evaporation of the solvent gave a crystalline residue (0.89 g). The residue was dissolved in ethanol–water (7:3; 25 mL), and PtO_2 (80 mg) was added. The mixture was stirred under an atmosphere of hydrogen for 6 h at room temperature. The catalyst was removed by filtration, and evaporation of the solvent gave crude 2-chloro-10,11-dihydro-10-(1-methyl-4-piperidyl)-5*H*-dibenzo[*a,d*]cyclohepten-10-ol (**17**; 0.83 g). The latter was dissolved in a mixture of concentrated HCl (1 mL) and acetic acid (15 mL), and this mixture was boiled for 30 min. The mixture was made alkaline by the addition of 2 N NaOH (150 mL) and then was extracted with ether (3 × 25 mL). The ether solution was washed with water (2 × 10 mL) and then extracted with 1 N HCl (3 × 10 mL). The acid extract was made basic by the addition of 2 N NaOH (75 mL) and was extracted with ether (3 × 50 mL). This ether solution was dried (Na_2SO_4) and evaporated. The residual oil (95 mg) was added to methylene chloride (50 mL) containing concentrated HCl (0.3 mL). Evaporation of this mixture gave an oil which crystallized on the addition of acetone (8 mL). Recrystallization from acetone gave pure **5**·HCl (35 mg, 6%), mp 272–274 °C dec (lit.[13] mp 140–141 °C for the free base). Anal. ($C_{12}H_{23}Cl_2N$) H, N; C: calcd, 70.00; found, 69.34.

2-Chloro-10-(4-methylpiperidino)-5*H*-dibenzo[*a,d*]cycloheptene (**8a**) was prepared from **10a** as described for the preparation of **4a** from **10a** except that 4-methylpiperidine was used in place of *N*-methylpiperazine. Crystallization from methanol gave pure **8a** (27%), mp 147–148 °C. Anal. ($C_{21}H_{22}ClN$) C, H, N.

2-Chloro-11-(4-methylpiperidino)-5*H*-dibenzo[*a,d*]cycloheptene (**8b**) was prepared from **10b** as outlined for the preparation of **4a** from **10a** except that 4-methylpiperidine was used in place of *N*-methylpiperazine. Crystallization from methanol gave pure **8b** (34%), mp 131–132 °C. Anal. ($C_{21}H_{22}ClN$) C, H, N.

2-Chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (**9**) was a gift from Sandoz Pharmaceuticals, Basel, and was a yellow solid with mp 201–203 °C (lit.[10] mp 201–203 °C).

2-Chloro-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-10-one (**10a**). 2-Benzyl-5-chlorophenylacetic acid (**11a**; 14.2 g, 54.5 mmol) was added in small portions with stirring to polyphosphoric acid ($H_6P_4O_{15}$; 70 g) at 100 °C. After the addition was complete, the mixture was heated at 130 °C for 4 h. After the mixture cooled, water (200 mL) was added, and the mixture was extracted with methylene chloride (3 × 100 mL). The methylene chloride solution was washed with saturated $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the organic solvent gave a solid residue (12.3 g). Recrystallization from ethanol gave pure **10a** (9.1 g, 69%), mp 144–146 °C (lit.[20] mp 146–147 °C).

2-Chloro-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-11-one (10b) and 2-Chloro-5*H*-dibenzo[*a*,*d*]cyclohepten-11-yl 2-(4-Chlorobenzyl)phenylacetate (16). 2-(4-Chlorobenzyl)-phenylacetic acid (**11b**; 13.0 g, 49.9 mmol) was added in small portions with stirring to polyphosphoric acid ($H_6P_4O_{15}$; 70 g) at 100 °C. The mixture was then heated at 135 °C for 1 h. After the mixture cooled, ice–water (600 mL) and chloroform (200 mL) were added. The aqueous layer was separated and extracted with chloroform (2 × 200 mL). The combined chloroform solutions were washed with 1 N NaOH and dried (Na_2SO_4). Evaporation of the chloroform gave an oil (11.5 g). Crystallization with benzene–cyclohexane (1:10) gave **16** (3.1 g, 26%), mp 134–136 °C. Recrystallization from ethanol gave pure **16**, mp 136–137 °C. Anal. ($C_{30}H_{22}Cl_2O_2$) C, H.

Evaporation of the benzene–cyclohexane mother liquors from the crystallization of **16** and crystallization of the residue from ethanol–acetonitrile (2:1) gave pure **10b** (4.4 g, 36%), mp 106–107 °C (lit.[20,21] mp 110 and 103–105 °C).

10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-one (10c) was prepared from **11c** as described for the preparation of **10a** from **11a**. Recrystallization from ethanol gave pure **10c** (44%), mp 68–71 °C (lit.[26] mp 75–76 °C).

2-Benzyl-5-chlorophenylacetic Acid (11a). Using a method outlined earlier,[22] a mixture of 2-benzoyl-5-chloracetic acid (**13a**; 25.0 g, 91.0 mmol), NaOH (20.0 g, 0.500 mol), hydrazine hydrate (29.5 g of 85% $N_2H_4 \cdot H_2O$, 0.50 mol), and diethylene glycol (70 mL) was heated to 120 °C for 1 h. The temperature was then raised to 190 °C for 2 h, during which water was removed by distillation. After cooling, the reaction mixture was diluted with water (250 mL) and was made acidic by the slow addition of concentrated HCl (50 mL). The precipitate was removed by filtration, and recrystallization from water–ethanol gave pure **11a** (16.0 g, 68%), mp 104–111 °C (lit.[20] mp 115–116 °C).

2-(4-Chlorobenzyl)phenylacetic acid (11b) was prepared from **13b** as described for the preparation of **11a** from **13a**. Recrystallization from water–ethanol gave pure **11b** (73%), mp 139–140 °C (lit.[21] mp 141–142 °C).

2-Benzylphenylacetic acid (11c) was prepared from **13c** as described for the preparation of **11a** from **13a**. Recrystallization from isopropyl ether gave pure **11c** (95%), mp 89–91 °C (lit.[20,22,26] mp 92–93, 89–90, and 95–95.5 °C).

5-Chloro-1-phenylindene (12a). Crude 5-chloro-1-phenyl-1-indanol (**15a**; 88.0 g, 0.360 mol) was dissolved in benzene (200 mL) and *p*-toluenesulfonic acid (2.1 g, 12 mmol) was added. The mixture was boiled for 4 h, during which water was removed using a water trap. After cooling, the mixture was diluted with ether (400 mL). The ether solution was washed with 1 N NaOH (2 × 200 mL) and saturated brine (2 × 50 mL), dried (Na_2SO_4), and evaporated. Recrystallization of the residue from methanol gave pure **12a** (25.2 g, 31%), mp 56–57 °C. Anal. ($C_{15}H_{11}Cl$) C, H, Cl.

1-(4-Chlorophenyl)indene (12b) was prepared from crude **15b** as described for the preparation of **12a** from **15a**. Recrystallization from methanol gave pure **12b** (63%), mp 64–65 °C [lit.[27] bp 135 °C (0.1 mm)]. Anal. ($C_{15}H_{11}Cl$) C, H, Cl.

1-Phenylindene (12c) was prepared from crude **15c** as described for the preparation of **12a** from **15a**. Recrystallization from methanol gave pure **12c** (82%), mp 25–28 °C [lit.[27] bp 108 °C (0.1 mm)].

2-Benzoyl-5-chlorophenylacetic Acid (13a). As a modification of a previously described method,[22] CrO_3 (75.0 g, 0.750 mol) was added in portions with stirring to 5-chloro-1-phenylindene (**12a**; 50.3 g, 0.222 mol) in acetic acid (1.0 L) and water (25 mL) at 30–35 °C. External cooling with ice was necessary during the addition. After the addition, stirring was continued for 2 h at 30–35 °C. The volume of the reaction mixture was then reduced to 400 mL and more water (600 mL) was added. This mixture was extracted with ether (3 × 300 mL), and the ether was washed with water (2 × 50 mL). The ether solution was then extracted with 1 N NaOH (4 × 250 mL), and this aqueous solution was made acidic with concentrated HCl (100 mL). The precipitated crystals were collected by filtration, and recrystallization from ethyl acetate–cyclohexane gave pure **13a** (28.2 g, 46%), mp 138–139 °C. Anal. ($C_{15}H_{11}ClO_3$) C, H, Cl.

2-(4-Chlorobenzoyl)phenylacetic acid (13b) was prepared from **12b** as described for the preparation of **13a** from **12a**. Recrystallization from ethyl acetate gave pure **13b** (86%), mp 160–161 °C (lit.[27] mp 162 °C). Anal. ($C_{15}H_{11}ClO_3$) C, H, Cl.

2-Benzoylphenylacetic acid (13a) was prepared as described for the preparation of **13a** from **12a**. Recrystallization from isopropyl ether gave pure **13c** (29%), mp 127–129 °C (lit.[27,28] mp 120 and 139 °C).

5-Chloro-1-indanone (14a). As reported earlier,[23] cyclization of 3-chlorohydrocinnamic acid[23] in methylene chloride using $AlCl_3$ catalyst gave **14a** (76%), recrystallized from hexane–ethyl acetate, mp 92–95 °C (lit.[23] mp 96–97 °C).

5-Chloro-1-phenyl-1-indanol (15a) was prepared by the Grignard addition of bromobenzene to **14a** as described in the following paragraph for the preparation of **15b** from **14b**. Crude **15a** (97%) was an oil and was used without further purification.

1-(4-Chlorophenyl)-1-indanol (15b). To Mg shavings (4.42 g, 0.182 g-atom) suspended in ether (50 mL) under nitrogen was added *p*-bromochlorobenzene (34.8 g, 0.182 mol) in ether (150 mL) at such a rate as to maintain gentle boiling. After the addition, stirring was continued for 1 h, and then 1-indanone (**14b**; 20.0 g, 0.151 mol) in ether (100 mL) was added. This mixture was stirred overnight at room temperature. Addition of 10% NH_4Cl (300 mL), separation of the ether layer, extraction of the aqueous phase with ether (2 × 200 mL), drying (Na_2SO_4) of the ether solution, and evaporation of the ether gave crude **15b** (36 g, 97%) as an oil. Crystallization of a small portion from pentane gave pure **15b**, mp 72–73 °C. Anal. ($C_{15}H_{13}ClO$) C, H, Cl.

1-Phenyl-1-indanol (15c) was prepared by the Grignard addition of bromobenzene to **14b** as described for the preparation of **15b** from **14b** and was used without further purification. Crude **15c** (100%) was an oil [lit.[22] bp 134–135 °C (1 mm)].

2-Chloro-10,11-dihydro-10-(4-pyridyl)-5*H*-dibenzo[*a*,*d*]-cyclohepten-10-ol (18). 2-Chloro-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]cyclohepten-10-one (**10a**; 5.3 g, 22 mmol) in tetrahydrofuran (50 mL) was slowly added with stirring to 4-lithiopyridine,[29] prepared in situ from 4-bromopyridine hydrobromide (8.4 g, 35 mmol) and 1.5 M butyllithium in ether (32 mL, 48 mmol) at −60 °C under nitrogen. Stirring was continued at −60 to −70 °C (dry ice–acetone) for 3 h, and then the mixture was allowed to warm to room temperature. Water (50 mL) was added, and the mixture was extracted with ether (2 × 100 mL). The ether was extracted with 1 N HCl (4 × 75 mL), and then this aqueous solution was made alkaline with 2 N NaOH (150 mL) and extracted with ether (2 × 100 mL). The ether was dried (Na_2SO_4) and evaporated. Recrystallization of the residue from ethanol gave **18** (0.95 g, 38% based on unrecovered **10a**), mp 246–247 °C (lit.[13] mp 250 °C). Anal. ($C_{20}H_{16}ClNO$) H, N; C: calcd, 74.64; found, 73.89.

The ether solution above, after extraction with the 1 N HCl, was dried (Na_2SO_4) and evaporated. Recrystallization from ethanol gave unreacted **10a** (3.4 g, 64%).

Animals and Materials for Biological Testing. Brain tissue was obtained from male Sprague–Dawley rats (200–400 g for caudate nuclei; 300–400 g for forebrain). [^{3}H]Clozapine was synthesized by Dr. A. Liebman and was a generous gift of Hoffmann-La Roche, Nutley, NJ. Unlabeled clozapine was donated by Sandoz Pharmaceuticals, Hanover, NJ. [^{3}H]Spiroperidol was purchased from New England Nuclear, Corp., Boston, MA. Unlabeled spiroperidol was a gift from Janssen Pharmaceuticals, Beerse.

Determination of Affinity for Clozapine Binding Sites. Fresh tissue (rat forebrain) was homogenized in 7 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 25 °C) containing $MgCl_2$ (5 mM). Homogenates were centrifuged (12000*g* for 15 min at 5 °C), and the pellet was resuspended in 7 volumes of Tris-HCl buffer. The tissue suspension was again centrifuged

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and the pellet resuspended in 14 volumes of Tris-HCl buffer. Aliquots (100 μL) containing approximately 0.3 to 0.4 mg of protein were added to incubation tubes. Total incubation volume was 500 μL and, in addition to tissue and varying amounts of drug compounds, contained the following: Tris-HCl buffer (50 mM, pH 7.7 at 25 °C), $MgCl_2$ (5 mM), ethanol (0.5 to 2%), and [^{3}H]clozapine (2.1 nM, specific activity = 24 Ci/mmol). Tissue samples were incubated both in the presence and absence of 1 μM atropine, a concentration sufficient to block [^{3}H]clozapine binding to muscarinic receptors.[17,30] [^{3}H]Clozapine binding in the presence of 10 μM clozapine was used to define nonspecific binding. Samples (prepared in triplicate) were incubated and filtered as described for [^{3}H]spiroperidol binding except that Tris-HCl buffer for filter washing contained 5 mM $MgCl_2$ and 0.5% ethanol and filters were presoaked in a 100 μM solution of unlabeled clozapine.

Determination of Affinity for Spiroperidol Binding Sites. Utilizing methods described previously,[19] frozen rat caudate nuclei were homogenized in 100 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 25 °C). Homogenates were centrifuged (50000*g* for 10 min at 5 °C) and the resulting pellet was resuspended in 100 volumes of Tris-HCl buffer. The tissue suspension was again centrifuged and the pellet resuspended in 150 volumes of Tris-HCl buffer containing the following: Tris-HCl buffer (50 mM, pH 7.7 at 25 °C), NaCl (120 mM), KCl (5 mM), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), ascorbic acid (0.1%), and pargyline (10 μM). The tissue suspension was warmed to 37 °C for 10 min and then returned to an ice bath. Various drug compounds and [^{3}H]spiroperidol (2.2 nM, specific activity = 35.9 Ci/mmol) were dissolved in 0.1% ascorbic acid. In some cases ethanol was added to aid solubility of the test compounds. The effect of this addition was controlled by adding equal amounts of ethanol to the assays used to determine total and nonspecific binding. Aliquots (1.8 mL) of the tissue suspension were added to incubation tubes together with varying amounts of drug compounds and [^{3}H]spiroperidol for a final volume of 2 mL. Samples (prepared in triplicate) were incubated for 20 min at 37 °C and then filtered over glass fiber filters (Whatman GF/C). Filters were washed with 15 mL of Tris-HCl buffer (50 mM, pH 7.7 at 25 °C), and the radioactivity remaining on the filters was determined by liquid scintillation counting techniques. The amount of [^{3}H]spiroperidol bound in the presence of 10 μM unlabeled spiroperidol is used to define nonspecific binding. Specific binding is defined as the difference between total and nonspecific binding.

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A Dopamine Receptor Model and Its Application in the Design of a New Class of Rigid Pyrrolo[2,3-*g*]isoquinoline Antipsychotics

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A hypothetical model of the interaction of antipsychotic drugs with the dopamine receptor is described. This three-dimensional molecular model has been developed on the basis of plausible intermolecular interactions between pharmacophoric groups of diverse types of antipsychotic drugs and postulated amino acid side chain substituents of the receptor protein. Three essential binding sites (one possibly required for antagonism) and one lipophilic auxiliary binding site are identified. The geometry is defined via the three-dimensional structures of drugs exhibiting receptor activity, including (*R*)-apomorphine, (+)-dexclamol, and molindone (whose crystal structure has been determined). A new conformationally rigid pyrrolo[2,3-*g*]isoquinoline derivative has been designed to conform to the receptor model. The compound (±)-1 (2,6-dimethyl-3-ethyl-4,4a,5,6,7,8,8a,9-octahydro-4a,8a-*trans*-1*H*-pyrrolo[2,3-*g*]isoquinolin-4-one; Ro 22-1319) exhibits potent antipsychotic-like activity. The activity is stereospecific, residing in the (-) enantiomer, predicted and confirmed by X-ray crystal structure analysis of (-)-1·HCl to have the 4a*R*,8a*R* absolute configuration.

The methodology of drug design could be greatly improved if receptors and their mode of interaction with active substances were known in precise molecular detail. Such information could be used, for example, to design conformationally defined structures in which pharmacophoric groups are oriented in the proper spatial arrangement for optimal receptor interaction. Compounds with this three-dimensional complementarity to a receptor should show greater potency, exhibit higher specificity, and have fewer effects at other receptors than conformationally flexible structures. Aspects of this ideal of drug design have been approached in the synthesis of enzyme inhibitors starting from the solid-state structures of enzyme–substrate complexes obtained through X-ray crystallography[1] and in the preparation of intercalating substances on the basis of the Watson–Crick model of DNA and the crystal structures of DNA model fragments.[2]

The molecular features of important pharmacologic receptors, however, are presently unknown beyond their pharmacological and biochemical classification[3] and their

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