Chloro-Substituted, Sterically Hindered 5,11-Dicarbo Analogues of Clozapine as Potential Chiral Antipsychotic Agents[1]

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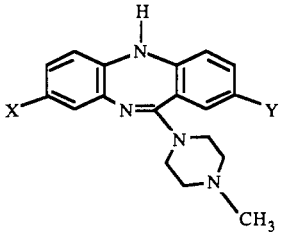
Variable-temperature proton nuclear magnetic resonance studies have shown that 5-(2-propylidene)-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene, a 5,11-dicarbo analogue of the atypical neuroleptic agent clozapine [8-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4]diazepine], exists as thermally stable configurational isomers. The presence of the 2-propylidene group at C-5 on the 5*H*-dibenzo[*a*,*d*]cycloheptene moiety did not interfere greatly, as compared to clozapine, with the in vitro affinity of this 5,11-dicarbo analogue of clozapine for muscarinic and dopamine D-1 and D-2 binding sites in rat brain. Since the presence and position of a chloro substituent on the 5*H*-dibenzo[*b*,*e*][1,4]diazepine moiety have a marked influence on the respective binding affinities of 1,4-diazepines related to clozapine, chloro-substituted 5,11-dicarbo analogues of clozapine were prepared in order to further examine structure-activity relationships. Evaluation of these analogues for binding to muscarinic and dopamine binding sites in comparison with clozapine and other 5*H*-dibenzo[*b*,*e*][1,4]diazepine analogues of clozapine shows that the dopamine D-1 and D-2 receptor affinities of both the 5-(2-propylidene)-5,11-dicarbo analogue and its corresponding distal-chloro derivative, 2-chloro-5-(2-propylidene)-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene, are retained. Because of the susceptibility to acid-catalyzed hydrolysis of these tertiary enamines, however, these compounds serve only as model compounds for their structure-activity evaluation. Since the proximal nitrogen atom of the piperazine ring is redundant for biological activity, 5-(2-propylidene)-10-(1-methyl-4-pyridyl)-5*H*-dibenzo[*a*,*d*]-cycloheptene and its 2-chloro derivative are excellent candidates for resolution into enantiomers as a means to separate antimuscarinic and antidopaminergic activity, respectively, associated with only a single stereoisomer.

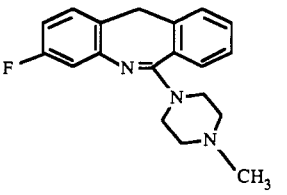
The high-dose, atypical neuroleptic agent clozapine [8-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4]-diazepine, **1a**] is an effective antipsychotic agent,[2] but its



1a, X = Cl; Y = H

b, X = Y = H

c, X = H; Y = Cl



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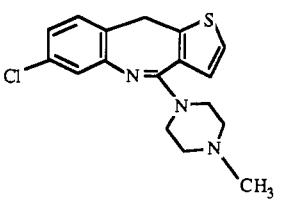
therapeutic use has been hampered by incidences of blood toxicity (agranulocytosis).[2] Several clozapine analogues have been evaluated, but at present, no substitute compound with a clozapine-like profile that is also free from side effects has been found,[3] and it may be speculated whether the presence of heteroatoms of clozapine is the origin of some of its toxicological problems. Indeed, attempts to develop other heterocyclic isosteres of clozapine as antipsychotic drugs seem to have been unsuccessful because of unexpected toxicity or unacceptable side effects. Development of fluperlapine (NB-106-689, **2**) had to be abandoned because clinical trials revealed some cases of agranulocytosis.[4] The thiophene analogue, tilozepine (NT 104-252, **3**), showed promising properties as an antipsychotic agent, but clinical trials had to be discontinued because **3** produced epileptiform seizures.[5] Flumezapine (LY 120363, **4**), another thiophene isostere of clozapine, was found to be a potent blocker of the dopamine D-2 and serotonin S-2 receptors,[6] but further development has not been reported. Further, in the 4-methyl-4*H*-thieno[3,4*b*] analogue (**5**) of clozapine, the cataleptogenic properties



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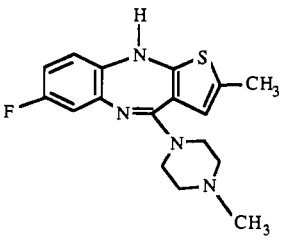

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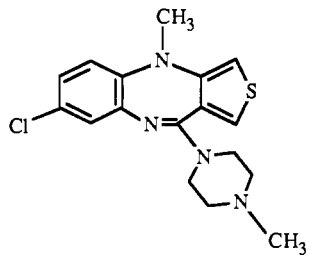

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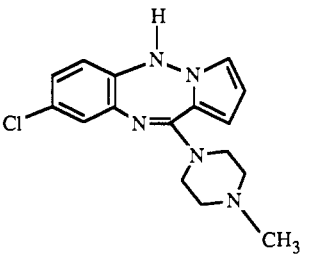

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were twice those of clozapine.[7] The pentanitrogen analogue **6** (Sandoz 200-125) was found to have a potential for pulmonary phospholipidosis in man,[8] and pentiapine

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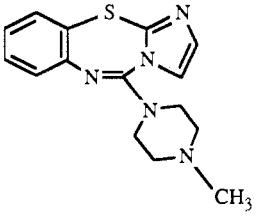
Table I. Affinity of Clozapine and Its Analogues for Binding Sites in Rat Brain

compd	IC_{50} ± SEM, nM[a] muscarinic ([^{3}H]QNB)	dopaminergic D-1 ([^{3}H]SCN 23390)	dopaminergic D-2 ([^{3}H]spiperone)
	Dibenzo[*b*,*e*][1,4]diazepines		
1a[b]	51 ± 8 (2)	192 ± 37 (5)	2770 ± 380 (7)
1b[b]	39 ± 11 (2)	895 ± 704 (2)	>10000 (2)
1c[b]	55 ± 18 (2)	63 ± 17 (2)	218 ± 28 (3)
	5-Methylenedibenzo[*a*,*d*]cycloheptenes		
9a	248 ± 73 (2)	51 ± 18 (2)	300 ± 143 (3)
9b[b]	150 ± 40 (2)	21 ± 1 (2)	305 ± 54 (4)
9c	330 ± 95 (3)	8 ± 5 (3)	27 ± 8 (2)
	5-(2-Propylidene)dibenzo[*a*,*d*]cycloheptenes		
10a	1750 ± 650 (3)	443 ± 185 (3)	5000 ± 1550 (3)
10b[b]	415 ± 15 (2)	540 ± 169 (3)	4750 ± 550 (2)
10c	297 ± 84 (3)	133 ± 29 (3)	>10000 (2)

[a] An IC_{50} value is the concentration of the compound necessary to displace 50% of specific radioligand binding. SEM is the standard error of the mean for the number of individual experiments, given in parentheses, conducted with triplicate determinations. [b] Data from ref 11.

(CGS-10746B, **7**) seems to induce receptor supersensitivity in mice.[9]



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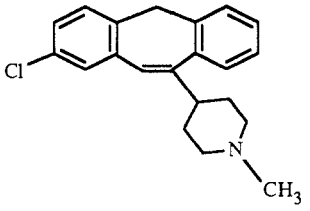

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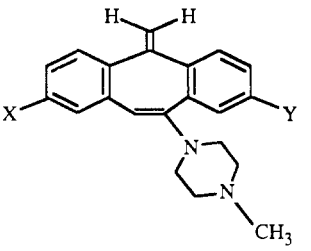
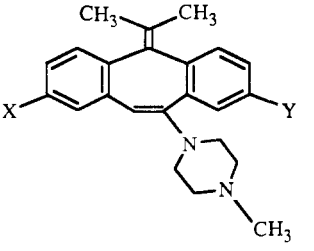
As alternate modifications of the clozapine structure, we earlier demonstrated that three of the four nitrogen atoms of clozapine (**1a**) can be replaced with carbon atoms while retaining a clozapine-like receptor binding profile.[10] Thus, the carbocyclic analogue **8** of clozapine [2-chloro-10-(1-methyl-4-piperidyl)-5*H*-dibenzo[*a*,*d*]cycloheptene] binds twice as potently to nonmuscarinic [^{3}H]clozapine binding sites in the rat forebrain as does clozapine while being 50 times less anticholinergic.[10]

In addition, conformational analysis of 5,11-dicarbo analogues of clozapine has shown that **8** and its 10-(4-methylpiperazino) analogue exist as nonplanar conformational enantiomers which are not resolvable because of the low activation energy for inversion of the seven-membered carbocyclic ring. In order to achieve resolution into stable configurational enantiomers of a derivative of **8** and thus the possible separation of desired biological properties into one enantiomer and of unwanted side effects into the other, one requirement is that the barrier of inversion of the seven-membered ring be sufficiently high so as to prevent thermal racemization. A recent dynamic proton nuclear magnetic resonance (^{1}H NMR) study[11] showed that the exocyclic 5-methylidene derivative **9b** has an activation energy of 19 kcal mol^{-1} for ring inversion, just below that considered to be necessary for separation into configurational enantiomers.[12] By an increase in the steric hin-

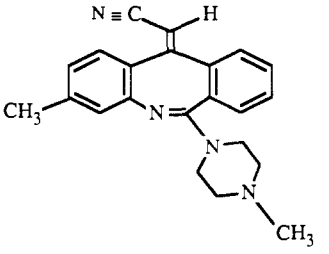


9a, X = Cl; Y = H
b, X = Y = H
c, X = H; Y = Cl



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

drance between the exocyclic substituent and the peri-hydrogen atoms at C-4 and C-6, the energy barrier to inversion is increased, and compound **10b** showed an activation energy >23 kcal mol^{-1} at 160 °C. That biological activity may be retained when a bulky substituent is present on C-5 of the seven-membered ring was demonstrated in that **10b** retains about half of the dopamine D-1 ([^{3}H]SCH 23390)[13] and D-2 ([^{3}H]spiperone)[14] receptor binding activity as compared to that of clozapine (**1a**) (Table I) and in that the (*Z*)-cyanomethylene derivative **11** of clozapine is reported to retain 42% of the antiapomorphine activity of **1a** in the rat on oral administration, while being only one-tenth as sedating.[15]



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Because of the sensitivity of D-2 binding affinities due to the presence or position of an aromatic chloro substituent in the clozapine series **1a–c**,[16] it became of interest to examine the effect of a chloro substituent on the binding affinities of the sterically hindered 5,11-dicarbo analogues of clozapine. We now report the syntheses and the muscarinic[17] and dopamine D-1[13] and D-2[14] receptor binding affinities of the distal and proximal chloro derivatives **9a,c** and **10a,c** of 5-methylene- and 5-(2-propylidene)-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene (**9b** and **10b**) and compare the binding affinities of **9a–c** and **10a–c** with those of clozapine (**1a**), deschloroclozapine[18] (**1b**), and isoclozapine[18] (**1c**).

Results

Synthesis. The preparation of 2-chloro-5-methylene-10(and -11)-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclo-

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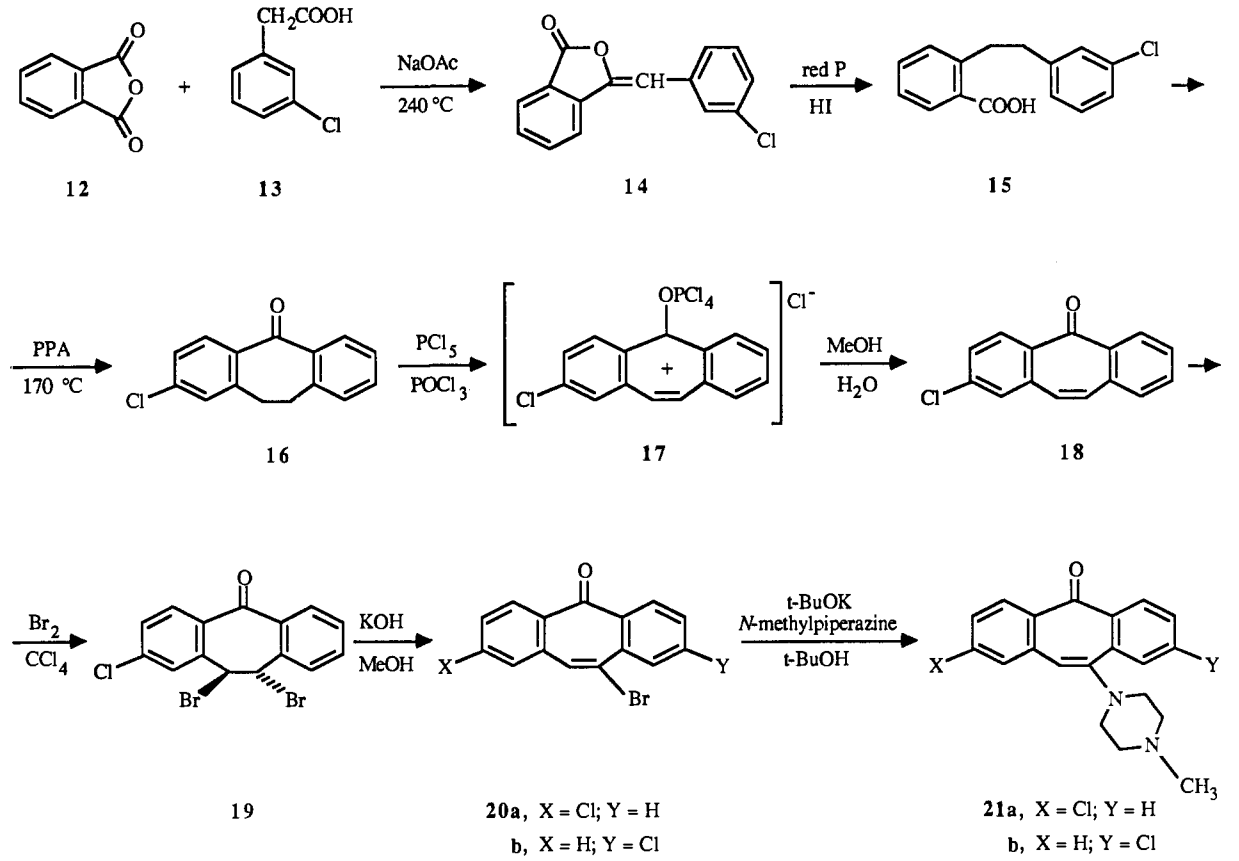
Scheme I

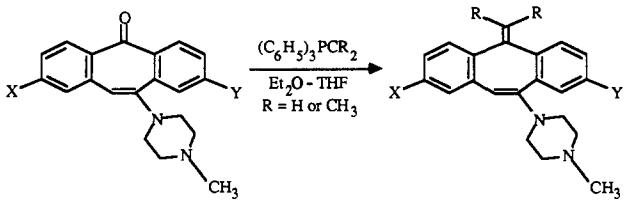


12 13 14 15 16 17 18 19

20a, X = Cl; Y = H
b, X = H; Y = Cl

21a, X = Cl; Y = H
b, X = H; Y = Cl

Scheme II



21a, X = Cl; Y = H
b, X = H; Y = Cl

9a, X = Cl; Y = R = H
c, X = R = H; Y = Cl
10a, X = Cl; Y = H; R = CH_3
c, X = H; Y = Cl; R = CH_3

heptene (**9a,c**) and 2-chloro-10(and -11)-(4-methylpiperazino)-5-(2-propylidene)-5*H*-dibenzo[*a,d*]cycloheptene (**10a,c**) is shown in Schemes I and II, the last synthetic step being the Wittig reaction of a triphenylphosphonium ylide with the intermediate ketones, 2-chloro-10(and -11)-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]cyclohepten-5-one (**21a,b**) (Scheme II). The preparation of these ketones (Scheme I) began with the condensation of phthalic anhydride (**12**) with (*m*-chlorophenyl)acetic acid (**13**), utilizing a reported procedure[19,20] and yielding 3-chlorobenzalphthalide[20] (**14**). The latter was reduced with red phosphorous in boiling 57% hydroiodic acid[20] to 2-[2-(*m*-chlorophenyl)ethyl]benzoic acid[20] (**15**). Cyclization of **15** to 2-chloro-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-one[20] (**16**) was accomplished by heating **15** in polyphosphoric acid (PPA) at 170 °C. The introduction of the additional double bond into the seven-membered ring of **16** by a method outlined earlier[21] gave 2-chloro-5*H*-dibenzo[*a,d*]cyclohepten-5-one[22] (**18**). This latter conversion proceeds by way of a dibenzotropylium chloride intermediate (**17**), a bright red, hygroscopic solid, which when treated with methanol–water gave **18**. Addition of bromine to **18** in carbon tetrachloride gave *trans*-2-chloro-10,11-dibromo-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-one (**19**), the configuration of **19** on the basis of the mechanism of the bromination reaction.[23] Treatment of **19** with methanolic sodium hydroxide gave a mixture of 10- and 11-bromo-2-chloro-5*H*-dibenzo[*a,d*]cyclohepten-5-one (**20a,b**). The presence of both **20a** and **20b** as reaction product was confirmed by carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy, but the estimation of the relative amounts of each isomer was not possible since the signals of **20a** and **20b** in the ^1H NMR spectrum of the mixture overlapped. Separation by thin-layer or column chromatography was not successful, but **20b** was isolated from the reaction mixture by crystallization. The structure of the isolated isomer was established as **20b** by two-dimensional correlated (COSY) and nuclear Overhouse effect (NOE) ^1H NMR experiments.

For preparation of pure samples of 2-chloro-10(and -11)-(4-methylpiperazino)-5*H*-dibenzo[*a,d*]cyclohepten-5-one (**21a,b**), no advantage is gained by isolation of pure samples of **20a** and **20b**. When either pure **20b** or a mixture of **20a** and **20b** in *tert*-butyl alcohol was treated with *N*-methylpiperazine and potassium *tert*-butoxide,

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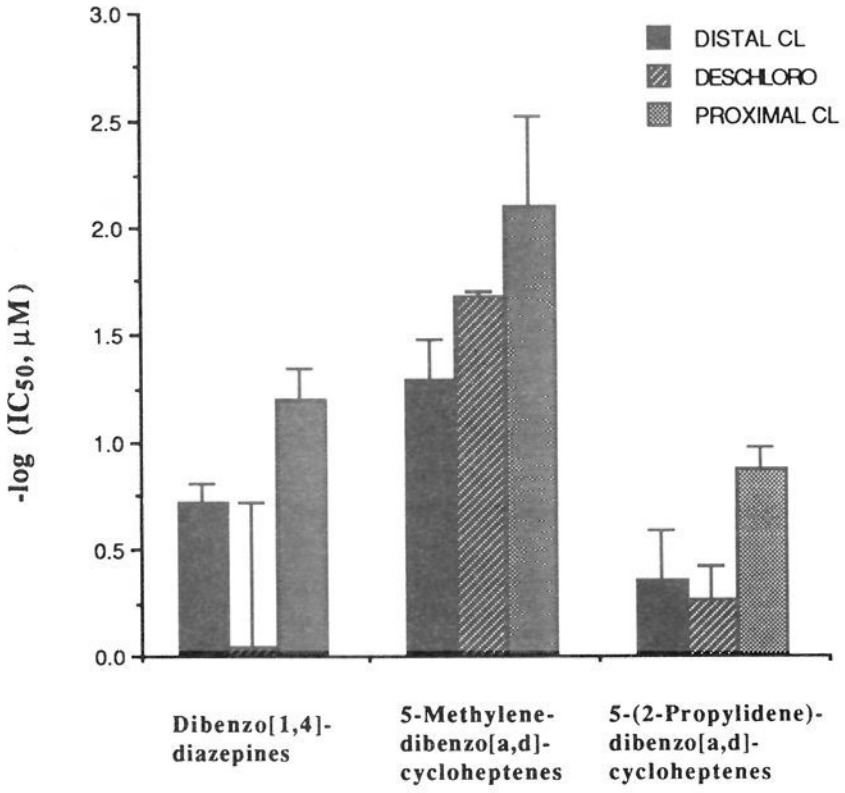
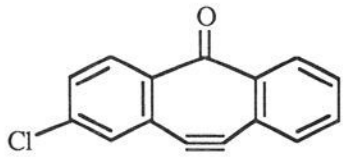


Figure 1. Relative dopamine D-1 binding activity of dibenzo[1,4]diazepines, 5-methylenedibenzo[*a*,*d*]cycloheptenes, and 5-(2-propylidene)dibenzo[*a*,*d*]cycloheptenes as shown by a comparison of their IC_{50} values, the concentration necessary to displace 50% of [^{3}H]SCH 23390 from sites in rat brain. Dibenzo[1,4]-diazepines: dist-Cl, clozapine (**1a**); des-Cl, deschloroclozapine (**1b**); prox-Cl, isoclozapine (**1c**). 5-Methylenedibenzo[*a*,*d*]cycloheptenes: dist-Cl, **9a**; des-Cl, **9b**; prox-Cl, **9c**. 5-(2-Propylidene)dibenzo[*a*,*d*]cycloheptenes: dist-Cl, **10a**; des-Cl, **10b**; prox-Cl, **10c**.

both **21a** and **21b** were formed, in a ratio of 1.5 to 1. Presumably the reaction proceeds by dehydrobromination of **20a** and **20b** to the alkyne intermediate **22** and then



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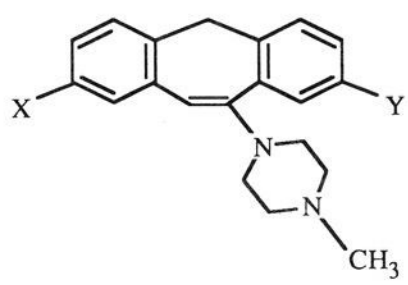
nucleophilic addition of *N*-methylpiperazine to either C-10 or C-11 of **22**. Separation of **21a** and **21b** was achieved by a combination of column chromatography and recrystallization, and the structures of **21a** and **21b** were established on the basis of COSY and NOE ^{1}H NMR spectroscopy. Reactions of the ketones **21a** and **21b** with respective ylides prepared from methyltriphenylphosphonium bromide and isopropyltriphenylphosphonium iodide were successful (Scheme II), but the overall conversions were low. Column chromatography was needed to separate the reaction products from the starting ketones **21a** and **21b**, but the latter could be recovered and were used in subsequent reactions.

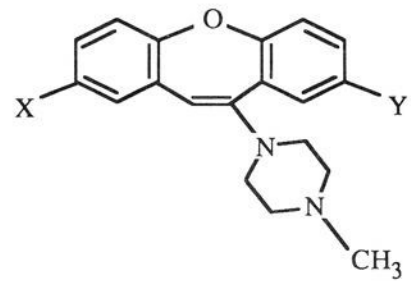
Nuclear Magnetic Resonance Studies. For establishment of the structure of the single pure substance isolated after dehydrobromination of **19** as either 10- or 11-bromo-2-chloro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**20a** or **20b**), use was made of the observation that in the isolated pure substance the vinylic proton at C-10 or C-11, occuring at 7.76 ppm among the aromatic proton signals, is the only singlet in the 400-MHz spectrum. The signals due to the C-1 and C-9 protons were assigned on the basis of a ^{1}H NMR COSY experiment. Saturation of the vinyl proton signal caused an NOE enhancement of the C-9 proton signal whereas the intensity of the C-1 proton signal remained unchanged. Thus, the vinyl proton was located at C-10, and structure **20b** was assigned to the isolated isomer. In confirmation of this assignment, saturation of the C-9 proton signal also caused an NOE enhancement of the vinylic proton signal, but irradiation with the C-1 proton frequency had no effect on the vinyl proton signal.

The structures of chloro-substituted isomers of 10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**21a**,**b**) were also established by COSY and NOE ^{1}H NMR experiments. Thus, for the isomer eluted first from a silica gel chromatography column, a sharp doublet at 7.41 ppm was assigned to the C-1 proton and a multiplet of 8.04 ppm to the C-9 proton on the basis of a ^{1}H NMR COSY experiment. Irradiation at the vinylic proton signal at 6.27 ppm gave a strong NOE enhancement to the C-1 proton signal whereas that of the C-9 proton remained unchanged. Thus the vinylic proton in this isomer is at the C-11 position (**21a**).

Biological Testing. In agreement with the in vitro biological activity of clozapine (**1a**) and other dibenzo[*b*,*e*][1,4]diazepine analogues of clozapine (**1b**,**c**)[10,24] and the related dibenzo[*a*,*d*]cycloheptene (**23a–c**)[10,24] and dibenz[*b*,*f*]oxepin analogues (**24a–c**), in the 5-methylene- and 5-(2-propylidene)dibenzo[*a*,*d*]cycloheptene series (**9a–c** and **10a–c**) (Table I), the presence of a chlorine atom in the position distal to the piperazino ring (**9a** and **10a**) has no effect on the affinity for dopamine D-2 binding sites. The presence of a chlorine atom in the proximal position (**9c** and **10c**) increases binding to both dopamine D-1 and D-2 sites except for **10c**, which is virtually inactive in displacing [^{3}H]spiperone from its binding sites in rat brain. The binding activity of the carbocyclic compounds in comparison to that of clozapine to the D-1 sites is shown in Figure 1. Within each series, the proximal chloro-substituted analogues **1c**, **9c**, and **10c** were the most potent. However, the compounds in the deschloro series had remarkably high affinity for the dopamine D-1 binding sites with the deschloro 5-methylene derivative **9b** being more than twice as active as the distal chloro analogue **9a** and over 40 times as active as deschloroclozapine (**1b**).



23a, X = Cl; Y = H
b, X = Y = H
c, X = H; Y = Cl



24a, X = Cl; Y = H
b, X = Y = H
c, X = H; Y = Cl

The considerable potency of clozapine in blocking [^{3}H]QNB binding was markedly reduced in the carbocyclic analogues. By use of the ratios between blockade of [^{3}H]QNB binding and the [^{3}H]SCH 23390 binding, the selectivity for binding to the dopamine D-1 sites over the muscarinic sites can be estimated. This reveals that the carbocyclic analogues are 3–27 times more dopamine-selective than is clozapine.

Discussion

The main question to be addressed by this study is whether the receptor binding profile of clozapine is retained in the 2- and 8-chloro-substituted 5,11-dicarbo

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analogues of clozapine when a methylene or 2-propylidene moiety is introduced in the 5-position of the seven-membered ring. Since clozapine exhibits fairly weak receptor binding affinities compared to those of classical neuroleptics, the relatively low potencies of the new compounds still endow them with a clozapine-like receptor binding profile. The present investigation confirms that 5-methylidene- and 5-(2-propylidene)-substituted 2-chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene (**9a** and **10a**) do indeed have a clozapine-like binding profile with regard to the dopamine D-1 and D-2 and to the muscarinic cholinergic receptors. In addition, and in agreement with our previous work,[10,16,24] the replacement of two of the nitrogen atoms of clozapine substantially reduces the affinity for the muscarinic binding sites as measured by displacement of [^{3}H]QNB from frontal cortex preparations of rat brain. Recent pharmacological studies of clozapine have demonstrated that the D-1 receptor blocking properties of clozapine are related to its atypical neuroleptic profile.[25] For example, dopamine D-1 agonist induced grooming behavior in mice is antagonized by clozapine,[26] but chronic administration of clozapine fails to reduce supersensitivity of the dopamine D-2 receptor in the rat.[27] Studies of the electrical activity in neocortex and hippocampus of rabbits indicate that concurrent blockade of both D-1 and D-2 receptors is required for neuroleptic action,[28] and it has been demonstrated that the lack of extrapyramidal side effects (EPS) of clozapine is not due to its anticholinergic properties.[29] Since both selective antagonists of the dopamine D-1 and D-2 receptors can induce catalepsy in mice,[30,31] it may be speculated that the absence of extrapyramidal side effects with clozapine is rather the result of a complex interaction of both the D-1 and D-2 receptor types. The fact that the D-1 and D-2 receptor blockade is retained in the 5,11-dicarbo analogues suggests that these compounds may have clinical properties similar to those shown by clozapine, including its low propensity to induce EPS.

Dynamic ^{1}H NMR studies with the deschloro derivative **10b**[11] clearly indicate the possibility of resolving the 5-(2-propylidene)dibenzo[*a*,*d*]cycloheptene analogues into their configurational enantiomers. Because of the susceptibility to acid-catalyzed hydrolysis of these tertiary enamines, however, these analogues serve only as model compounds for structure–activity relationship evaluation. Since also the 1-nitrogen atom of the piperazino ring is redundant for biological activity,[10] the 5-(2-propylidene) derivative of the 1-methyl-4-piperidyl compound 8 and its deschloro derivative are obvious candidates for attempted synthesis and resolution into enantiomers. This work is now in progress.

Experimental Section

Melting points were taken in open capillary tubes and are corrected. Solvent evaporations were done at reduced pressure by using a water pump. Proton nuclear magnetic resonance (^{1}H NMR) spectra used for characterization of products were obtained in chloroform-*d* by using a JEOL FX-90Q or Bruker AM-400 spectrometer operating at 90 and 400 MHz, respectively. The carbon-13 nuclear magnetic resonance spectrum (^{13}C NMR) was obtained in chloroform-*d* by using a Bruker AC-200 spectrometer operating at 50 MHz. The ^{1}H NMR correlated spectroscopy (COSY) and nuclear Overhauser effect (NOE) experiments were done by using the Bruker AM-400 instrument. For measurement of NOE difference spectra, 1.0 Hz of exponential line broadening was used. For COSY spectra, the standard Bruker microprogram was used, and 256 1K spectra were recorded by using a 90° $-t_1$–40° pulse sequence. Sine-bell apodization, magnitude calculation, and symmetrization were employed. For all NMR spectra, chemical shifts are recorded as parts per million (ppm) downfield from tetramethylsilane. Combustion analyses were done by Galbraith Laboratories, Knoxville, TN, and agree to within 0.4% of the calculated values or unless otherwise noted.

Biological testing was done as previously reported.[11]

2-Chloro-5-methylene-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene (9a). *n*-Butyllithium (1.4 mL, 1.55 M in hexanes, 2.2 mmol) was added dropwise with stirring to methyltriphenylphosphonium bromide (0.69 g, 1.9 mmol) in dry ether (15 mL) followed by 2-chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**21a**) (0.50 g, 1.5 mmol) in tetrahydrofuran (15 mL). The solution was boiled for 6 h, cooled, and poured onto ice–water (50 mL). The organic phase was separated, and the aqueous phase was extracted with ether (3 × 25 mL). The combined organic phase and ether extracts were dried ($MgSO_4$), and evaporation gave a reddish tar (0.9 g) consisting of **9a**, **21a**, and triphenylphosphine oxide.

Column chromatography on silica gel (150 g) with ethyl acetate–absolute ethanol–triethylamine (100:1.0:0.25) as eluant gave some fractions containing only **9a**, moving as a colorless band just ahead of the bright yellow band of the starting ketone **21a**. Evaporation of these combined fractions gave a yellow oil (0.37 g). The oil was crystallized from ethyl acetate, and recrystallization (2×) from the same solvent gave **9a** (58 mg, 12%) as a light yellow solid: mp 119–121 °C; ^{1}H NMR (JEOL FX-90Q) δ 7.79–7.18 (m, 7, aromatic H), 6.06 (s, 1, C-11 H), 5.27–5.22 (d of d, 2, J = 2 Hz, C=CH_2), 2.95 (m, 4, C-2 and C-6 piperazino H), 2.55 (m, 4, C-3 and C-5 piperazino H), 2.34 ppm (s, 3, NCH_3). Anal. ($C_{21}H_{21}ClN_2$) C, H, Cl, N.

2-Chloro-5-methylene-11-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cycloheptene (9c) was prepared from 2-chloro-11-(4-methylpiperazino)-5*H*-bicyclo[*a*,*d*]cyclohepten-5-one (**21b**) and methyltriphenylphosphonium bromide as outlined above for the preparation of **9a** from **21a**. Column chromatography on silica gel and recrystallization from ethyl acetate gave **9c** (8%) as a light yellow solid: mp 109–111 °C; ^{1}H NMR (JEOL FX-90Q) δ 7.74 (m, 2, aromatic H), 7.26–7.19 (m, 5, aromatic H), 6.20 (s, 1, C-10 H), 5.25 (d of d, 2, J = 2 Hz, C=CH_2), 2.99 (m, 4, piperazino C-2 and C-6 H), 2.58 (m, 4, piperazino C-3 and C-5 H), 2.36 ppm (s, 3, NCH_3). Anal. ($C_{21}H_{21}ClN_2$) C, H, Cl, N.

2-Chloro-10-(4-methylpiperazino)-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (10a). *n*-Butyllithium (2.5 mL, 1.55 M in hexanes, 3.9 mmol) was added dropwise with stirring to isopropyltriphenylphosphonium iodide (2.5 g, 5.8 mmol) in dry ether (30 mL). The mixture was stirred and boiled for 3.5 h, and 2-chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**21a**) (1.00 g, 2.95 mmol) in ether (15 mL) was added with stirring. The mixture was stirred overnight at room temperature and then diluted with water (75 mL). After filtration, the organic layer was separated, and the aqueous phase extracted with ether (3 × 50 mL). The combined organic phases and ether extracts were dried ($MgSO_4$) and evaporated. The residual tar (1.2 g) was subjected to chromatography on silica gel (80 g) with ethyl acetate–absolute ethanol–triethylamine (100:1.0:0.25) as eluant. The product **10a** moved on the chromatographic column as a colorless band just ahead of the bright yellow band of the starting ketone **21a**. Fractions containing only **10a** were combined, and the solvent was evaporated. Crystallization of the residual oil from absolute ethanol and recrystallization from methanol gave **10a** (250 mg, 23%) as a white solid: mp 140–142 °C; ^{1}H NMR (JEOL FX-90Q) δ 7.74 (m, 2, aromatic H), 7.34–7.08 (m, 5, aromatic H), 6.02 (s, 1, C-11 H), 2.96 (m, 4, piperazino C-2 and C-6 H), 2.56 (m, 4, piperazino C-3 and C-5 H), 2.35 (s, 3, NCH_3), 1.69 (s, 3, =CCH_3),

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1.66 ppm (s, 3, =CCH_3). Anal. ($C_{23}H_{25}ClN_2$) C, H, Cl, N.

2-Chloro-11-(4-methylpiperazino)-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (10c) was prepared from 2-chloro-11-(4-methylpiperazino)-5*H*-bicyclo[*a*,*d*]cyclohepten-5-one (**21b**) and isopropyltriphenylphosphonium iodide as outlined above for the preparation of **10a** from **21a**. Column chromatography on silica gel, crystallization from absolute ethanol, and recrystallization from methanol gave **10c** (5%) as light yellow solid: mp 148–150 °C; ^1H NMR (Bruker AM-400) δ 7.69 (d, 1, J = 2.3 Hz, C-1 H), 7.30–7.10 (m, 6, aromatic H), 6.15 (s, 1, C-10 H), 2.99 (m, 4, piperazino C-2 and C-6 H), 2.61 (m, 4, piperazino C-3 and C-5 H), 2.37 (s, 3, NCH_3), 1.69 (s, 3, =CCH_3), 1.66 ppm (s, 3, =CCH_3). Anal. ($C_{23}H_{25}ClN_2$) C, H, Cl, N.

***trans*-2-Chloro-10,11-dibromo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (19).** Bromine (4.8 g, 0.030 mol) in carbon tetrachloride (10 mL) was added dropwise with stirring to a suspension of 2-chloro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**18**), mp 158–159 °C (lit.[22] mp 159–160 °C) (6.00 g, 24.9 mmol), in carbon tetrachloride (25 mL). The mixture was stirred at room temperature for 4 h and then washed with aqueous sodium thiosulfate. The washings were extracted with ether (3 × 50 mL), and the ether extracts were combined with the carbon tetrachloride solution. The dried ($MgSO_4$) organic solution was evaporated, and recrystallization of the residue from ethanol gave **19** (6.6 g, 66%): mp 165–167 °C dec; ^1H NMR (JEOL FX-90Q) δ 8.13–8.04 (m, 2, C-4 and C-6 H), 7.56–7.40 (m, 5, aromatic H), 5.72 ppm (d, 2, C-10 and C-11 H). Anal. ($C_{15}H_9Br_2ClO$) C, H, Br, Cl.

11-Bromo-2-chloro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (20b). *trans*-2-Chloro-10,11-dibromo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**19**) (12.0 g, 30.0 mmol) and sodium hydroxide (3.6 g, 90 mmol) in methanol (250 mL) were boiled for 1.5 h. The solvent was evaporated and the solid residue mixed with water (100 mL). The resulting slurry was extracted with ether. Evaporation of the dried ($MgSO_4$) ethereal extracts gave a mixture (9.2 g, 96%) of 10-bromo-2-chloro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one (**20a**) and **20b** as a slightly yellow solid: mp 130–135 °C. (lit.[32] mp 127–129 °C). Separation of these isomers by chromatography was not successful, but recrystallization of a portion (1.0 g) from hexanes gave **20b** (0.50 g, 48% from **19**): mp 142–143 °C; ^1H NMR (Bruker AM-400) δ 8.09 (d, 1, J = 1.9 Hz, C-1 H), 7.93 (d, 1, J = 8.5 Hz, C-6 H), 7.84 (d, 1, J = 8.5 Hz, C-4 H), 7.76 (s, 1, C-10 H), 7.61–7.58 (m, 1, C-7 H), 7.56–7.54 (m, 1, C-8 H), 7.51–7.47 (m, 1, C-3 H), 7.44–7.40 ppm (m, 1, C-9 H); ^{13}C NMR δ 192.93 (carbonyl C), 138.63, 138.15, 137.52, 135.72, 134.68, 132.74, 132.05, 130.82, 130.72, 130.02, 129.93, 129.51, 128.88, 123.42 ppm. Anal. ($C_{15}H_8BrClO$) C, H, Br, Cl.

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2-Chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (21a). *N*-Methylpiperazine (7.6 g, 0.076 mol) and potassium *tert*-butoxide (4.4 g, 0.039 mol) were added with stirring to a mixture of 10-bromo- and 11-bromo-2-chloro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5-one (**20a** and **-b**) (12.0 g, 37.5 mmol) suspended in *tert*-butyl alcohol (70 mL), and the stirred mixture was boiled for 4 h. The solvent was evaporated, and the residual tar was mixed with water (200 mL). The resulting slurry was extracted with methylene chloride (5 × 200 mL). Evaporation of the dried ($MgSO_4$) methylene chloride solution gave a mixture (10 g, 79%) of **21a** and 2-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one (**21b**) in a ratio of 1.5 to 1, respectively (^1H NMR), as an orange-brown solid. Chromatography on silica gel (500 g) using ethyl acetate–absolute ethanol–triethylamine (100:1.0:0.25) as eluant gave, in the combined earliest fractions (7 × 100 mL), **21a** contaminated with a small amount of **21b**. Evaporation of these fractions and recrystallization of the residue from absolute ethanol gave **21a** (1.5 g, 12%) as bright yellow needles: mp 110–113 °C; ^1H NMR (Bruker AM-400) δ 8.05–8.02 (m, 1, C-9 H), 7.88–7.85 (m, 1, C-6 H), 7.76 (d, 1, J = 9.7 Hz, C-4 H), 7.62–7.51 (m, 2, C-8 and C-7 H), 7.41 (d, 1, J = 2.0 Hz, C-1 H), 7.30–7.26 (m, 1, C-3 H), 6.27 (s, 1, C-11 H), 3.04–2.93 (m, 4, piperazino C-2 and C-6 H), 2.65–2.55 (m, 4, piperazino C-3 and C-5 H), 2.38 ppm (s, 3, NCH_3). Anal. ($C_{20}H_{19}ClN_2O$) C, H, Cl, N.

2-Chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (21b). Middle fractions (5 × 100 mL) from the chromatography outlined above contained mixtures of **21a** and **21b**. The combined latest fractions (9 × 100 mL) contained mostly **21b** and were evaporated. Recrystallization of the residue from absolute ethanol gave **21b** (0.90 g, 7%) as bright yellow needles: mp 130–132 °C; ^1H NMR (Bruker AM-400) δ 8.03 (d, 1, J = 2.1 Hz), 7.88 (s, 1), 7.78 (s, 1), 7.43 (m, 4), 6.43 (s, 1, C-10 H), 2.95 (m, 4, piperazino C-2 and C-6 H), 2.65 (m, 4, piperazino C-3 and C-5 H), 2.39 ppm (s, 3, NCH_3). Anal. ($C_{20}H_{19}ClN_2O$) C, H, Cl, N.

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Registry No. **9a**, 124380-92-3; **9c**, 124380-93-4; **10a**, 124380-94-5; **10c**, 124380-95-6; **18**, 3973-55-5; **19**, 124380-98-9; **20a**, 124381-00-6; **20b**, 124380-99-0; **21a**, 124380-96-7; **21b**, 124380-97-8; methyltriphenylphosphonium bromide, 1779-49-3; isopropyltriphenylphosphonium iodide, 24470-78-8; *N*-methylpiperazine, 109-01-3.

Potential Antitumor Agents. 59. Structure–Activity Relationships for 2-Phenylbenzimidazole-4-carboxamides, a New Class of "Minimal" DNA-Intercalating Agents Which May Not Act via Topoisomerase II

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A series of substituted 2-phenylbenzimidazole-4-carboxamides has been synthesized and evaluated for in vitro and in vivo antitumor activity. These compounds represent the logical conclusion to our search for "minimal" DNA-intercalating agents with the lowest possible DNA-binding constants. Such "2-1" tricyclic chromophores, of lower aromaticity than the structurally similar 2-phenylquinolines, have the lowest DNA binding affinity yet seen in the broad series of tricyclic carboxamide intercalating agents. Despite very low in vitro cytotoxicities, several of the compounds had moderate levels of in vivo antileukemic effects. However, the most interesting aspect of their biological activity was the lack of cross-resistance shown to an amsacrine-resistant P388 cell line, suggesting that these compounds may not express their cytotoxicity via interaction with topoisomerase II.

Although high DNA binding affinity correlates positively with in vitro cytotoxicity for several series of DNA-intercalating agents,[1,2] this property is also thought to be the factor limiting the penetration of such drugs into multi-

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