SYNTHESIS OF NOVEL GUANIDINES AS POTENTIAL PSYCHOACTIVE AGENTS

by

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A THESIS

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Dr. John F. W. Keana

In recent years, study has been devoted to the nature and function of neuroreceptors. The sigma receptor is a pharmacologically well characterized binding site found in mammalian brain and other organs. The term sigma comes from SKF 10,047, a drug which causes characteristic psychotomimetic symptoms. IC_{50} studies have shown SKF 10,047 and PCP to bind to a site distinct from the previously characterized PCP receptor and opioid receptors. The discovery that 1,3-di-ortho-tolylguanidine has strong and specific affinity for the sigma site has prompted the synthesis of various similar molecules. This thesis details the synthesis of N,N'-dicyclohexylguanidine and N"-substituted analogues for use in characterizing the sigma binding site more fully.

ACKNOWLEDGEMENTS

I would like to thank Dr. John Keana, for his invaluable guidance, and for making his laboratory available for this research. Special thanks are due to Drs. James Long and Donald Taylor, who served on my thesis comittee. In addition, I express my appreciation to Dr. Michael Scherz and the other members of the Keana laboratory for their constant assistance with practical problems. The research was supported in part by grants from the National Institute of Mental Health and from Cambridge Neurosciences Research Inc. A summer fellowship was provided by the Oregon Affiliate of the American Heart Association. Finally, I would like to thank Dr. Geraldine Richmond for encouraging me to consider research in chemistry.

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CHAPTER I

INTRODUCTION

It is only recently that enough has been learned about the structure and function of the nervous system that novel chemicals may be designed to interact with sites known to regulate specific physiological responses. Attention has recently been directed towards characterization of neural receptors.

Receptors are membrane bound proteins or protein complexes which transduce miniscule chemical signals into physical or chemical responses.[1] A common mechanism of action is the binding of a small molecule called a ligand, which changes the properties of the receptor enough to allow the flow of ions (charged particles) or molecules across the membrane. If the ligand binding the receptor causes a response, it is called an agonist. If it inhibits a receptor's response to some other ligand, it is termed an antagonist.

The sigma receptor was first postulated to explain the nervous body movements and 'canine delirium' caused by the drug SKF 10,047 (allylmetanormazine) in spinally severed dogs.[2] It was originally described as an opioid receptor, but opioid receptors are antagonized by naloxone and the sigma receptor is not.[3,4]

Brain receptors are present in amounts of only

femtomoles to picomoles (10^{-15} to 10^{-12} moles) per milligram of total brain protein. In addition, they usually lose their activity when isolated from cell membranes. As a result, initial characterization of receptors is usually based on the relative affinity of a receptor for various ligands, as determined by IC_{50} tests.

Biologically useful binding is an equilibrium process, where the fraction of receptors bound by a ligand varies with the concentration of that ligand. The IC_{50} test involves the treatment of brain homogenate with a fixed concentration of radioactive ligand which is known to bind tightly and selectively to a particular receptor, followed by exposure to various concentrations of an unknown compound. The concentration required to displace 50% of the radioactive substance is the IC_{50} value. A smaller IC_{50} value indicates a potent ligand for the particular receptor bound by the radioactive substance.

Mammalian brain tissue contains many different receptors and ligands often cross-react with several of them. For example, PCP (phenylcyclidine) interacts with the PCP receptor and the sigma receptor,[5] the NMDA (N-methyl-D-aspartate) receptor,[6] and even interferes with cocaine binding.[7] SKF 10,047 was the first known ligand for the sigma receptor, but (+)-3-PPP [N-n-propyl-3-(3-hydroxy-phenyl) piperidine][8] and DTG [N,N'-di-ortho-tolylguanidine][9] were subsequently found to bind very strongly to the sigma site and show very little tendency to cross-react with

others.

DTG's unique properties make it an exciting "lead" compound which may form the basis for novel therapeutic agents whose actions would be mediated through the sigma receptor. Only a few chemicals with unexpected pharmacological activity are discovered each year, so this group of compounds is well worth further investigation. Interestingly, a group of DTG derivatives have been used as photolabels, which are allowed to reach a binding equilibrium with the sigma receptor and are then irreversibly bound to their receptor by light-activation of reactive functional groups. Once irreversibly bound, these compounds aid the isolation and identification of the sigma receptor. This method has been used to isolate several polypeptides which may be part of a larger sigma receptor complex.[10,11]

The extreme potency and specificity of DTG (1) prompted the design of similarly structured molecules as potential neuroactive drugs. N,N'-dicyclohexylguanidine (DChG) (2) is similar to DTG except its carbon rings are saturated and are therefore less rigid. In this current work, a number of N"-substituted compound derivatives of DChG were undertaken to investigate the effects on sigma receptor binding when all three guanidine nitrogens have a substituent, something not previously tried.

All of the trisubstituted derivatives were formed by coupling DCC with the corresponding primary amine, a

standard method.[12] The disubstituted guanidine (2) was synthesized by fusing cyanogen bromide with two equivalents of cyclohexylamine.[13,14]

Syntheses of the N"-methyl[15] (3), N"-allyl (4), N"-hydroxy (5),[16] N"-octyl (6) and N"-amino (7) derivatives of dicyclohexylguanidine were undertaken. Samples of DChG and its methyl and allyl derivatives were obtained in pure form, the latter two as hydrochloride salts. Crude hydrochloride salts of the hydroxy and octyl derivatives were also synthesized, but time constraints prevented their complete purification. Reacting hydrazine with DCC gave the double addition product bis-N,N'-dicyclohexylguanidine (8), rather than the desired N,N'-dicyclohexyl-N"-aminoguanidine, even when excess hydrazine was used. However, analytical and spectral data indicate that the bis-DChG compound did not cyclize and eliminate cyclohexylamine as the bis-N,N'-diphenylguanidine eliminates aniline.[17] Most of these compounds were found in the literature, but the N"-allyl (4), N"-octyl(6) and bis (8) derivatives were not.

Compounds synthesized and purified to constant melting point had ^{1}H and decoupled ^{13}C NMR and IR spectra taken and were sent for elemental analysis as an additional criteria of purity. Five mg samples of each pure substance were sent to the Vollum Institute in Portland and to Cambridge Neurosciences Laboratory for IC_{50} binding assays.

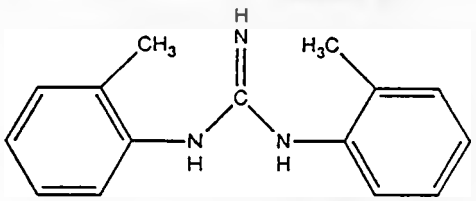
CHAPTER 2

EXPERIMENTAL METHODS

General. Reactions were done under N_2 gas and stirred magnetically. Liquid amines were injected neat via hypodermic syringe, after standard purification procedures. Et_2O and THF (tetrahydrofuran) were distilled from benzophenone ketyl prior to use. Elemental analyses were performed by Desert Analysis (Tucson, AZ). NMR spectra were taken in $[^2H]$-CH_3OH (HCl salts) or $[^2H]$-$CHCl_3$ on a General Electric QE-300. Shifts are relative to residual proton peaks in the solvent. An extended relaxation time was necessary to accent the guanidine carbon peaks on ^{13}C NMR. IR spectra were taken from KBr pellets on a Nicolet 5DXB Fourier Transform Infrared (FT-IR) spectrometer, in order to confirm a guanidine stretching band in the region 1600-1640 cm^{-1} and the absence of a band at 2130cm^{-1}.[12]

FIGURE 1

Structure of DTG and DCC



(1) DTG

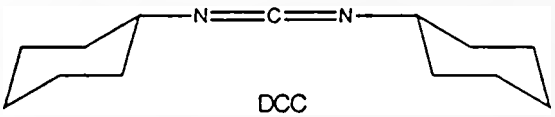
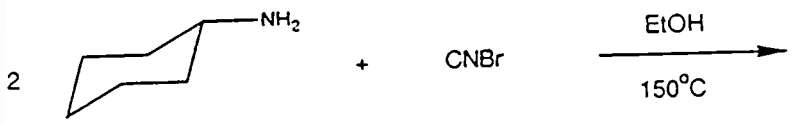
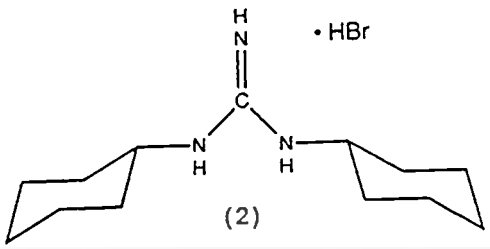


FIGURE 2

Synthesis of N,N'-dicyclohexylguanidine (DChG)





(2)

FIGURE 3

Synthesis of N"-methyl-DChG

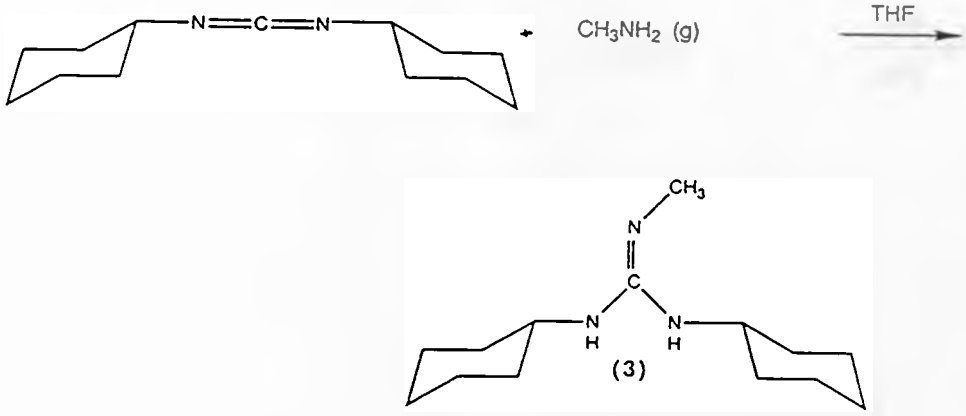


FIGURE 4

Synthesis of N"-allyl-DChG

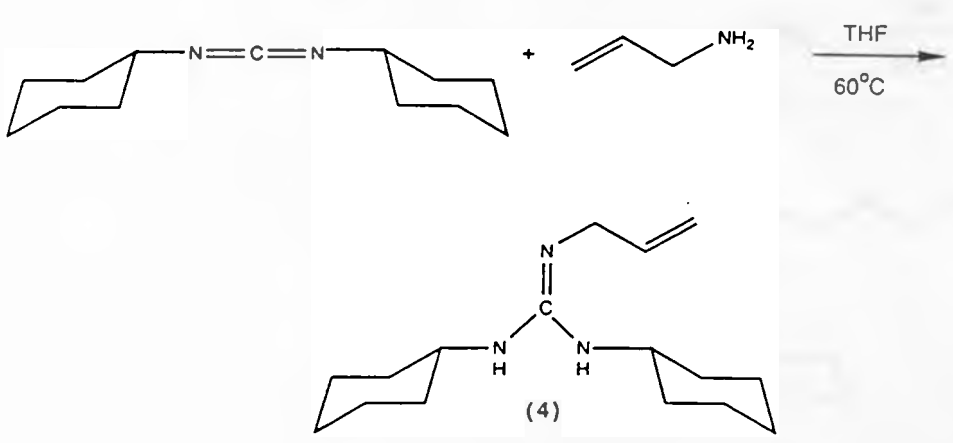


FIGURE 5

Synthesis of N"-hydroxy-DChG

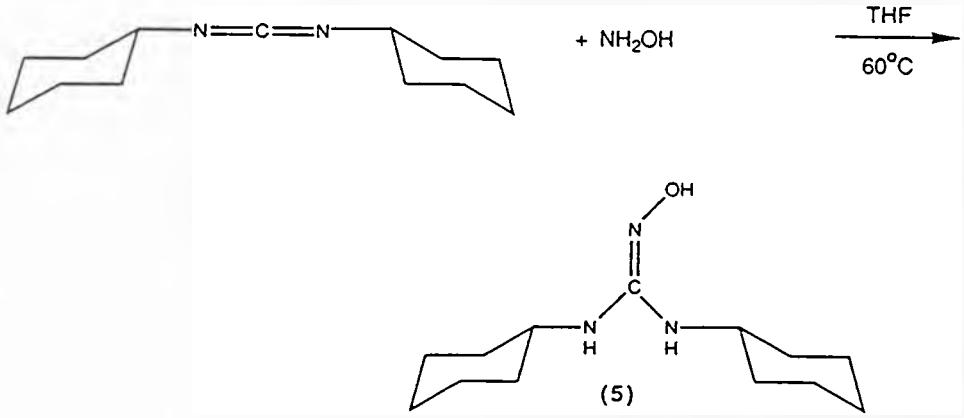


FIGURE 6

Synthesis of N"-octyl-DChG

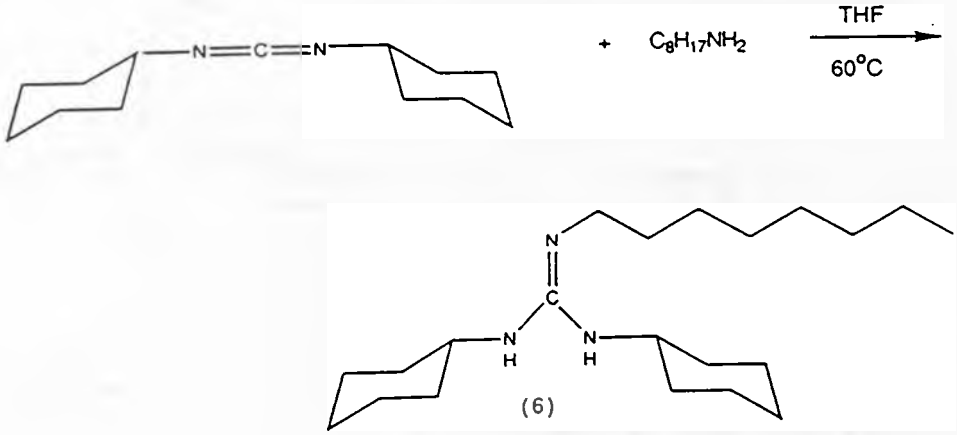


FIGURE 7

Formation of N"-amino-DChG

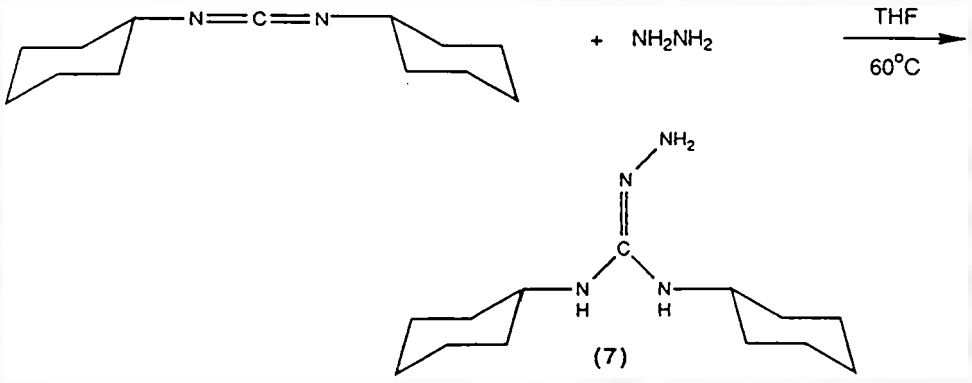
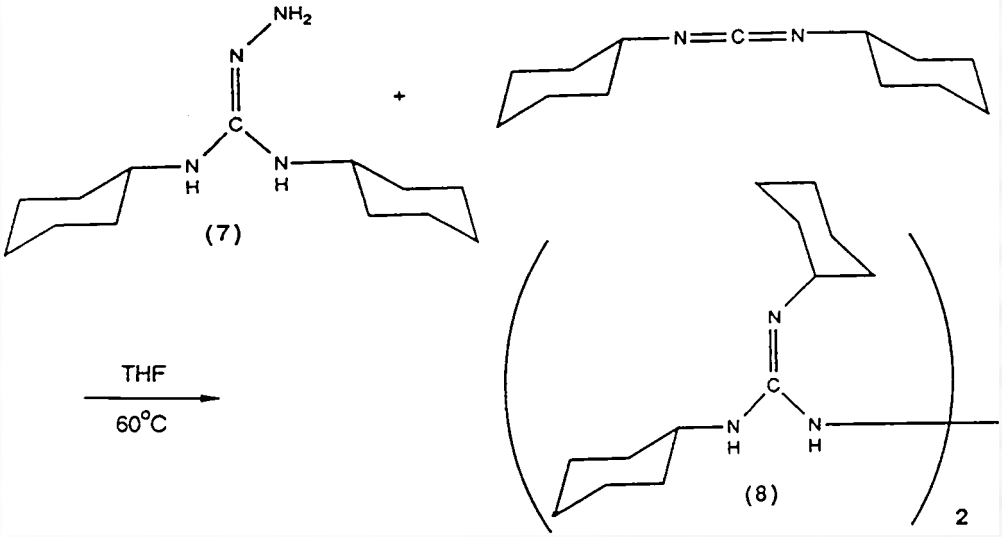


FIGURE 8

Formation of bis-N,N'-dicyclohexylguanidine



N,N′-Dicyclohexylguanidine (2).[13,14] Cyanogen bromide (447 mg, 4.22 mmol) was dissolved in absolute ethanol (4 mL), cooled in ice and added to a solution of cyclohexylamine (800 mg, 7.8 mmol) in ethanol (1.5 mL). Heating at 150°C for 15 minutes gave a clear glass, which was dissolved in ethanol (15 mL), filtered and treated with 1N NaOH, causing a white precipitate which was collected and dried. Recrystallization (EtOH/H_2O 1:1) produced white crystals of (2), (256 mg, 23%, M.P. 181-182°C). ^1H NMR ([^2H]-$CHCl_3$) *d* 3.25 (quint, 2, N-bearing cyclohexyl), 2.1-0.9 (m, 22, cyclohexyl). ^{13}C NMR ([^2H]-$CHCl_3$) *d* 156.69 (s, guanidine), 50.05 (s, N-bearing cyclohexyl), 33.77. 25.68 and 24.92 (singlets, cyclohexyl). IR shows peaks at 1665 and 1615 cm^{-1}. Elem. Analysis ($C_{13}H_{25}N_3$) Theor. C 69.91, H 11.28, N 18.81. Found C 69.99, H 11.29, N 18.84.

N,N′-Dicyclohexyl-N"-methylguanidine hydrochloride (3).[15] Methylamine gas was slowly bubbled for several hours into a solution of DCC (dicyclohexylcarbodiimide) (596 mg, 2.89 mmol) in THF. The mixture was evaporated and vacuum dried for 43 hours at room temperature, giving a viscous yellow oil (687 mg). Filtering in chloroform removed dark impurities which appeared upon standing. Evaporation and solution in ethanol (30 mL) followed by addition of conc. HCl (0.37 mL, 1.5 eq.) gave the HCl salt, which was vacuum dried. Precipitation from ethanol with ether gave a white

precipitate and red oil. Two recrystallizations (EtOH/Et_2O 5:7) gave thick white prisms of (3) visible under magnification (179 mg, 23%, M.P. 278-279°C). ^1H NMR ([^2H]-CH_3OH) *d* 4.96 (s, 3, methyl), 2.0-1.0 (m,22, cyclohexyls). ^{13}C NMR ([^2H]-CH_3OH) *d* 153.5 (guanidine), 51.0 (N-bearing cyclohexyl), 32.44 (methyl), 27.17, 24.80 and 24.69 (cyclohexyls). IR shows a characteristic band at 1620 cm^{-1}. Elem. Analysis ($C_{14}H_{28}N_3$) Theor. C 61.40, H 10.31, N 15.34. Found C 60.71, H 10.20, N, 14.81.

N,N'-dicyclohexyl-N"-allylguanidine hydrochloride (4). DCC (496 mg, 2.41 mmol) and allylamine (10 mL, 130 mmol) was stirred 3.5 hours at 50°C. The resulting yellow mixture was evaporated, dissolved in chloroform and again evaporated to remove allylamine. The remaining viscous oil was dissolved in ethanol (10 mL) and acidified with ethanolic HCl (0.25 mL conc. HCl in 10 mL EtOH). Evaporation with additional absolute ethanol followed by three reprecipitations (1.0 mL ethanol in an ether chamber gave fine white powder (4) (33.1 mg, 15%, M.P. 238-241°C). ^1H NMR ([^2H]-CH_3OH) *d* 5.98-5.78 (m, 1, - CH_2-C<u>H</u>=CH_2), 5.24-5.18 (t, 2, -C<u>H_2</u>-CH=CH_2), 3.91 (d, 2, -CH_2-CH=C<u>H_2</u>), 3.52-3.39 (m, 2, N-bearing cyclohexyl), 1.95-1.07 (m, 22, cyclohexyls). ^{13}C NMR ([^2H]-CH_3OH) *d* 154.5 (guanidine), 134.3 (allyl), 117.1 (allyl), 52.6 (N-bearing cyclohexyl), 44.5 (terminal allyl), 33.9 26.2 and 26.0 (cyclohexyls). IR shows an absorption band at 1615 cm^{-1} in the guanidine range of 1600-1640 cm^{-1}.

Elem. Analysis ($C_{16}H_{30}N_3Cl$) Theor. C 64.08, H 10.08, N 14.01, Found C 64.14, H 10.28, N 13.96.

N,N'-dicyclohexyl,N"-hydroxyguanidine.[16] A mixture of DCC (789 mg, 3.82 mmol) and hydroxylamine hydrochloride (248 mg, 3.57 mmol) in tetrahydrofuran (25 ml), was stirred with triethylamine (296 mg, 2.93 mmol) and refluxed for six hours, at which time iodine revealed two spots (Rf=0.05,0.90) showed on TLC with ethanol. The mixture was dissolved in diethyl ether (25 mL) and extracted with water (3 * 15 mL). Evaporation of the ether layer gave a yellow-white powder (549 mg, 2.29 mmol, 64%, M.P. 94-104^{o}C). Recrystallization in petroleum ether gave a waxy yellow powder (66 mg), but further purification was abandoned.

N,N'-dicyclohexylguanidine-N"-octylguanidine. DCC (1.25 g, 6.07 mmol) was dissolved in tetrahydrofuran (30 mL) and charged with octylamine (750 mg, 6 mmol). After refluxing three days, the mixture was filtered and diluted with THF (to 50 mL), acidified with 50 mmol of 0.1N HCl. The resulting solution was neutralized with NaOH pellets and with methylene chloride (4 * 25 mL). Evaporation gave a yellow oil (1.42 g, 71%) with a single spot on TLC (Rf=0.55 with ethanol). Solution in diethyl ether (50 mL) and dropwise addition of HCl saturated ether gave a fluffy white precipitate (1.37 g, 62%, M.P. 145-153^{o}C). Recrystallization (unheated ethanol in an ethyl acetate

chamber) gave white cubic crystals (20 mg, 1%, M.P. 240-249). Low yield prevented further purification.

Bis-N,N'-dicyclohexylguanidine (8).[17] DCC (1.03 g, 4.97 mmol) was dissolved in tetrahydrofuran (15 mL). Hydrazine (135 mg, 4.22 mmol) was added via syringe. Stirring 30 hours at room temperature produced a clear yellow mixture which was evaporated to a white powder (922 mg). Solution of this powder in diethyl ether, filtration and acidification with HCl (60 mL of saturated Et_2O) gave a yellow white precipitate (856 mg) Two recrystallizations (EtOH in an Et_2O chamber) gave white prisms (133 mg, 18%, M.P. 288-290^{o}C). The low percentage of nitrogen in the elemental analysis of this compound indicates that two equivalents of DCC have reacted to hydrazine, forming bis-N,N"-dicyclohexylguanidine rather than the desired N"-amino compound. Subsequent reactions with dropwise addition of DCC to a 5:1 excess of hydrazine were expected to favour a 1:1 addition of DCC, but gave a product with an proton NMR spectrum identical to that of (8) and were therefore not recrystallized. ^{1}H NMR ([^{2}H]-CH_3OH) *d* 3.21 (s, 4, N-bearing cyclohexyl), 2.0-1.5, (m, 44, cyclohexyls). ^{13}C NMR ([^{2}H]-CH_3OH) *d* 154.1 (guanidine), 51.7 (N-bearing cyclohexyl), 32.4 and 24.8 (cyclohexyls. IR shows eaks at 1639 and 1589 cm^{-1}. Elem. Analysis ($C_{26}N_6H_{50}Cl_2$: 1/2 H_2O) Theor. C 59.30, H 9.76, N 15.96, Found C 58.94, H 9.76, N 16.01.

Chapter III

RESULTS

After purification was complete, samples of (2), (3), and (4) were sent to Eckhard Weber at the Vollum Institute (Oregon Health Sciences University) and to Cambridge Neuroscience Laboratories for IC_{50} testing of the their ability to displace $[^3H]$-DTG and $[^3H]$-MK-801 (a receptor which specifically binds the PCP receptor). The results were as follows:

TABLE I

IC_{50} CONCENTRATIONS OF DICYCLOHEXYLGUANIDINES AGAINST KNOWN SIGMA AND PCP RECEPTOR LIGANDS IN MURINE BRAIN

Cmpd. No.	substituent	$[^3H]$-DTG	$[^3H]$-MK-801
(2)	hydrogen	71+/- 7 nM	>10,000 nM
(3)	methyl	237+/-56 nM	>10,000 nM
(4)	allyl	513+/-57 nM	>10,000 nM
(8)	n/a*	>10,000 nM	>10,000 nM

* This is a symmetrical diguanidine.

As expected, the compounds showed a moderate affinity for the sigma receptor and almost no affinity for the PCP receptor. The relative flexibility of the cyclohexyl rings as compared to the rigid shape of the ortho-tolyl groups of

DTG (1) probably makes the binding equilibrium unfavourable. Nevertheless, the weak binding does indicate that at least one conformation of the dicyclohexyl guanidine structure fits the sigma binding site. Current research is aimed at designing very rigid molecules which are locked into a single conformation, allowing more precise identification of the shape of the sigma binding site. Ultimately, identification of the nature of the sigma receptor and its ligands may lead to better understanding of mental illnesses and effective ways to treat them.

The results described in this thesis are included in an article currently accepted for publication by the Journal of Medicinal Chemistry.[18] This is the most recent of a number of articles by Keana, Weber and collaborators, which describe characteristics of the sigma receptor and guanidines which bind it.

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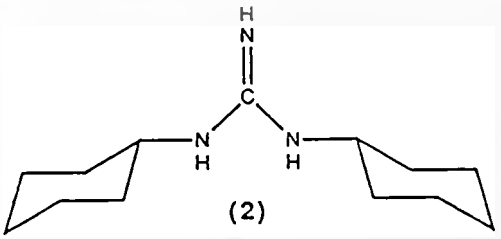
APPENDIX A: PROTON NMR

SPECTRUM 1

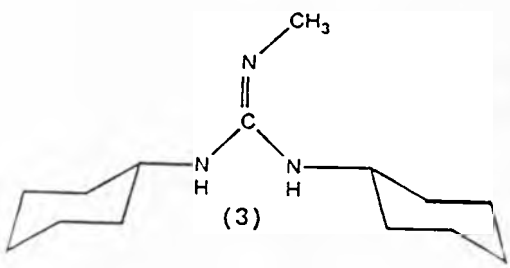
^{1}H spectrum of DChG



(2)

SPECTRUM 2

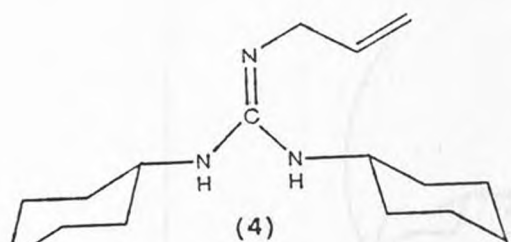
^{1}H spectrum of N"-methyl-DChG



(3)

SPECTRUM 3

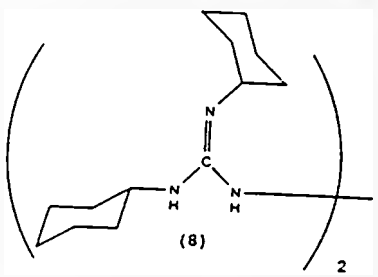
^1H spectrum of N"-allyl-DChG



(4)

SPECTRUM 4

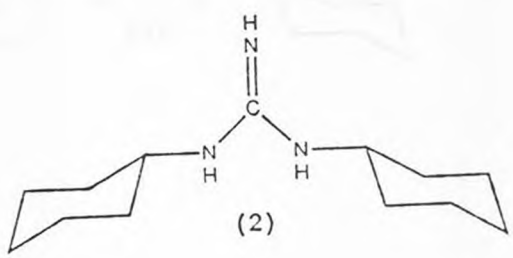
^{1}H spectrum of bis-DChG



APPENDIX B: CARBON NMR

SPECTRUM 5

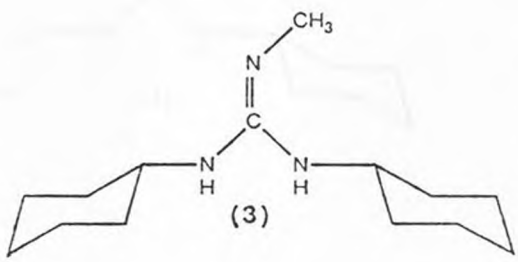
^{13}C spectrum of DChG



(2)

SPECTRUM 6

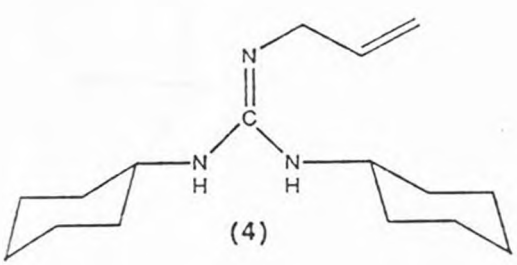
^{13}C spectrum of N"-methyl-DChG



(3)

SPECTRUM 7

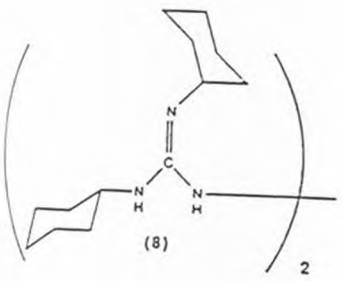
^{13}C spectrum of N"-allyl-DChG



(4)

SPECTRUM 8

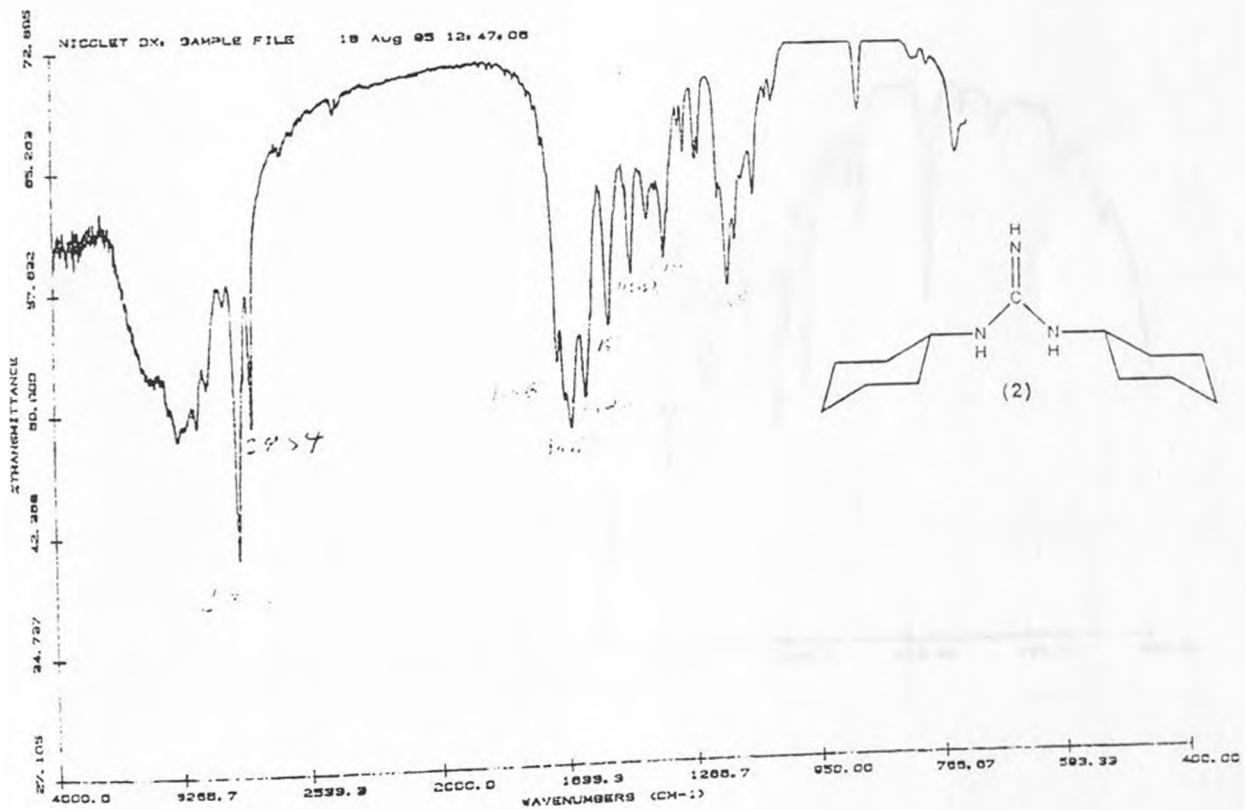
^{13}C spectrum of bis-DChG



APPENDIX C: INFRARED ABSORPTION

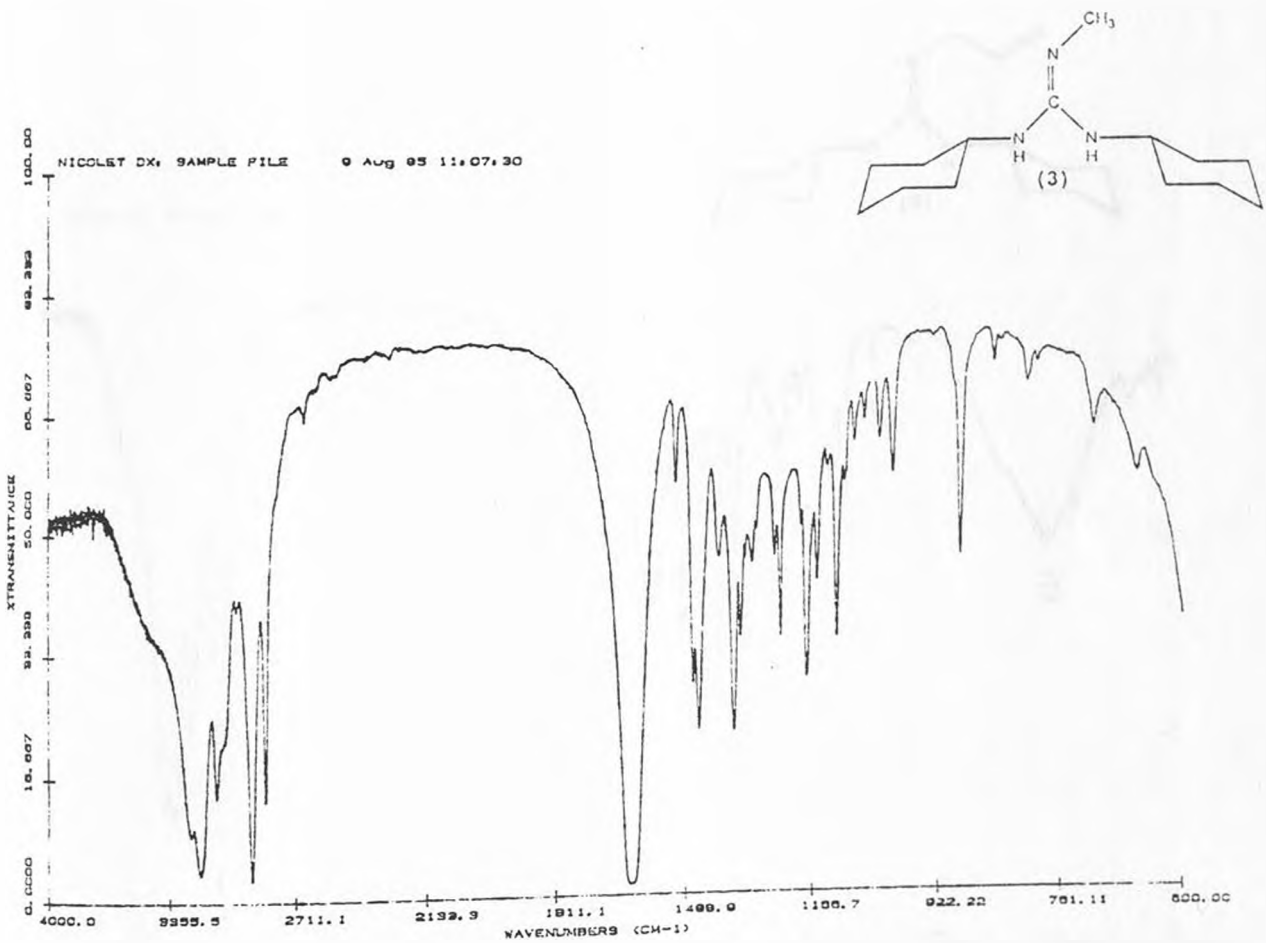
SPECTRUM 9

IR spectrum of DChG



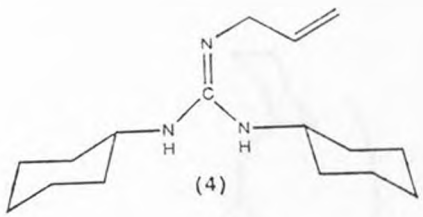
SPECTRUM 10

IR spectrum of N"-methyl-DChG

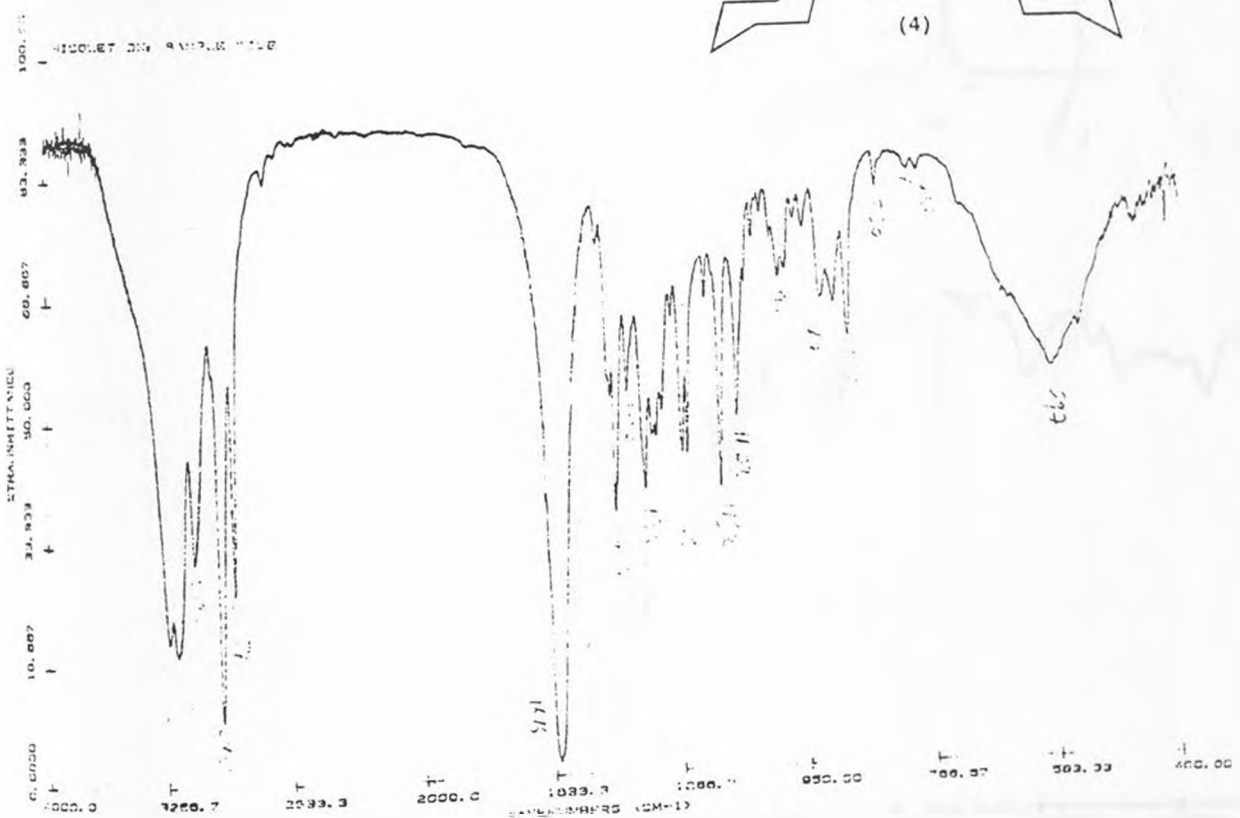


SPECTRUM 11

IR spectrum of N"-allyl-DChG



(4)



SPECTRUM 12

IR spectrum of bis-DChG