Health. Gifts of compounds from Dr. A. A. Manian were greatly appreciated.

References and Notes

(1) L. Levy, T. Tozer, L. D. Tuck, and D. Loveland, *J. Med. Chem.*, **15**, 989 (1972).
(2) F. H. Merkle, C. A. Discher, and A. Felmeister, *J. Pharm. Sci.*, **53**, 965 (1964).
(3) F. H. Merkle and C. A. Discher, *J. Pharm. Sci.*, **53**, 620 (1964).
(4) R. McCreery, *J. Pharm. Sci.*, **66**, 367 (1977).
(5) L. H. Piette, G. Bulow, and I. Yamazaki, *Biochim. Biophys. Acta*, **88**, 120 (1964).
(6) T. Akera and T. Brody, *Mol. Pharmacol.*, **4**, 600 (1968).
(7) I. S. Forrest and D. F. Green, *J. Forensic Sci.*, **17**, 592 (1972).
(8) H. Y. Cheng, P. H. Sackett, and R. L. McCreery, *J. Am. Chem. Soc.*, **100**, 962 (1978).
(9) T. Akera and T. Brody, *Biochem. Pharmacol.*, **21**, 1403 (1972).
(10) T. Akera and T. M. Brody, *Mol. Pharmacol.*, 6, 557 (1970).
(11) S. Ohnishi and H. M. McConnell, *J. Am. Chem. Soc.*, **87**, 2293 (1965).
(12) G. M. Gooley, H. Keyzer, and F. Setchell, *Nature (London)*, **223**, 80 (1969).
(13) T. Akera, C. Y. Lee, and T. M. Brody, *Biochem. Pharmacol.*, **25**, 1751 (1976).
(14) L. Piette and I. S. Forrest, *Biochim. Biophys. Acta*, **57**, 419 (1962).
(15) S. H. Snyder, I. Creese, and D. Burt, *Science*, **192**, 471 (1976).
(16) P. Seeman, T. Lee, M. Chau-Wong, and K. Wong, *Nature (London)*, **261**, 717 (1976).
(17) T. A. Grover, L. H. Piette, and A. A. Manian in "The Phenothiazines and Structurally Related Drugs", I. S. Forrest, C. J. Carr, and E. Usdin, Ed., Raven Press, New York, N.Y., 1974, p 561.
(18) M. Neptune and R. L. McCreery, *J. Med. Chem.*, **21**, 362 (1978).
(19) T. Koenig, R. Wielesek, L. Miller, and Y. So, *J. Am. Chem. Soc.*, **99**, 7061 (1977).

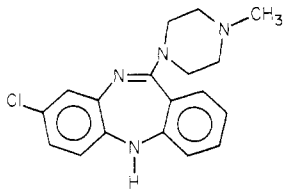
Synthesis and Biological Evaluation of Some 2-Amino-4-aryl-3*H*-1,5-benzodiazepine Analogues of Clozapine

Charles R. Ellefson,* Chi M. Woo, Arni Miller, and Janet R. Kehr

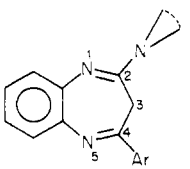
Searle Laboratories, Chicago, Illinois 60680. *Received January 23, 1978*

2-Amino-4-aryl-3*H*-1,5-benzodiazepines were prepared and evaluated for potential neuroleptic activity. Compound **6o** showed some activity in the four assays; however, the activity was not consistently observed among other members of the series. The data reflected that the structural modifications led to a decrease in activity relative to clozapine. It was apparent that the 1,5-benzodiazepine portion of clozapine is not responsible for its antipsychotic activity.

Clozapine (**1**), an example of a new class of neuroleptic



1



2

piperazinyldibenzoazepines,[1] is particularly significant because it lacks extrapyramidal side effects in man.[2] This has given renewed momentum to the search for new psychotropic drugs with diminished side effects compared to standard drugs such as chlorpromazine and haloperidol. Because the piperazinyldibenzoazepines such as **1** do not structurally resemble other clinically active antipsychotic agents, it was our intention to investigate whether a portion of the molecule might be responsible for its activity. It is interesting that there are both a 1,4- and a 1,5-benzodiazepine moiety contained within the framework of **1**. Because the 1,4-benzodiazepines are more familiar as antianxiety agents,[3] we have undertaken a study of some 2-amino-4-aryl-3*H*-1,5-benzodiazepine analogues of **1** (such as **2**) and have evaluated them for potential neuroleptic activity.

One theory of schizophrenia, the dopamine hypothesis,[4] suggests that the putative neurotransmitter, dopamine, mediates the observed disorders associated with schizophrenia and that the antipsychotic drugs act by inhibition of dopamine at the postsynaptic receptor. Even though the effects of **1** may not be mediated by blockade of dopamine receptors,[5] the 2-amino-1,5-benzodiazepines (**2**) do have a spatial relationship between the aromatic ring and the 2-amino substituent that is similar to the extended trans conformation[6] of dopamine. It was anticipated that the title compounds could interact at the dopamine receptor via that structural feature. The compounds were, therefore, also evaluated for action on dopaminergic systems.

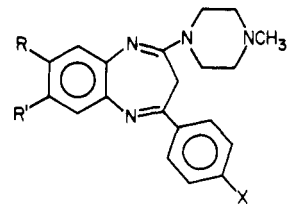
Screening for antipsychotic agents is complicated by the lack of suitable animal models and, therefore, has depended on assays that make comparisons with the observed effects of clinically active antipsychotic agents in animals.[7] The activity profile of clozapine does not resemble the more traditional neuroleptic agents,[5] however, and since it lacks extrapyramidal side effects in man[2] our goal was to find a compound with activities similar to **1** in the biological evaluation of **5**–**7**.

Chemistry. Synthesis of the title compounds was accomplished by displacement of a methylthioimino ether by secondary amines as outlined in Scheme I. Treatment of a 1,5-benzodiazepine-2-thione (**3**) with sodium hydride and methyl iodide in refluxing benzene afforded the methylthioimino ether, **4**, which was used without further purification. Displacement of the methylthio group was carried out in refluxing chloroform or toluene in the presence of excess amine and glacial acetic acid. The products have been classified according to the type of amine that was used for the displacement: **5** from *N*-methylpiperazine, **6** from 4-arylpiperidines, and **7** from some miscellaneous secondary amines. The most distinguishable spectral property of these amidines (**5**–**7**) was the UV spectrum. The UV absorptions and other data about these compounds are given in Tables I–III. General procedures for their preparation are given in the Experimental Section.

The thiolactam intermediates (**3**) were prepared similarly to literature methods[8,9] as outlined in Scheme II. Various substituted acetophenones were reacted with

0022-2623/78/1821-0952$01.00/0

Table I. 2-(4-Methylpiperazino)-3*H*-4-aryl-1,5-benzodiazepines

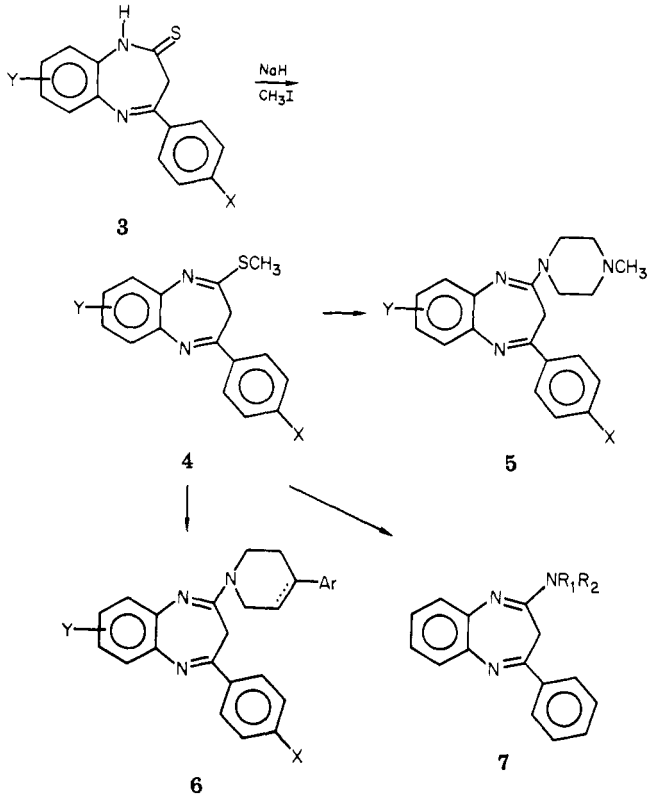


5

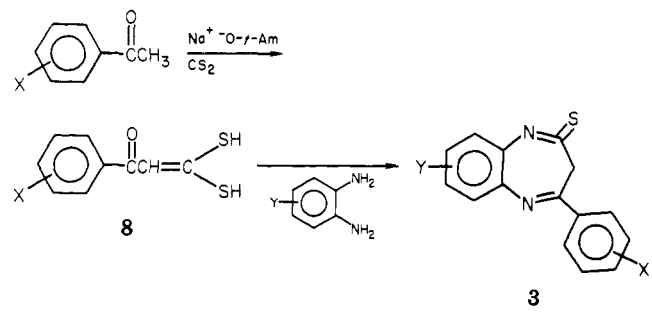
compd	R[a]	R′	X	% yield	mp, °C	recrystn solvent[b]	formula	analyses	λ max	ε max
5a				50	155-157	A	$C_{20}H_{22}N_4$	C, H, N	258 342	33400 4800
5b	Cl			34	125.5-126.5	A	$C_{20}H_{21}ClN_4$	C, H, N	262 356	37400 4800
5c	CH_3	CH_3		64	131.5-134.5	B	$C_{22}H_{26}N_4$	C, H, N	260 350	33400 5900
5d			Cl	42	155.5-157.5	C	$C_{20}H_{21}ClN_4$	C, H, N	260.5 348	39300 5700
5e			F	46	157-158	D	$C_{20}H_{21}FN_4$	C, H, N	259 341	34000 5000
5f			OCH_3	56	148.5-150	D	$C_{21}H_{24}N_4O$	C, H, N	265.5 338	38100 8000
5g			CH_3	36	129.5-132	D	$C_{21}H_{24}N_4$	C, H, N	260.5 345	39000 6400
5h	CH_3	CH_3	F	22	169-170	D	$C_{22}H_{25}FN_4$	C, H, N	262.5 345	34100 5800

[a] Blank spaces represent H. [b] A, MeOH; B, hexane; C, cyclohexane; D, EtOAc-hexane; E, $CHCl_3$-hexane; F, EtOAc; G, EtOH; H, *i*-PrOH.

Scheme I



Scheme II



sodium *tert*-amylate in the presence of carbon disulfide; acidification then produced the aryloyldithioacetic acids 8. The thio acids were characterized by two D_2O exchangeable protons in the ^1H NMR spectrum, one of which was extremely far downfield from Me_4Si (916 and 326 Hz for benzoyldithioacetic acid). A mixture of 8 and an *o*-phenylenediamine in refluxing xylene produced the 1,5-benzodiazepine-2-thiones 3. These products crystallized from the reaction solution and were suitable for further reaction after washing with xylene and drying.

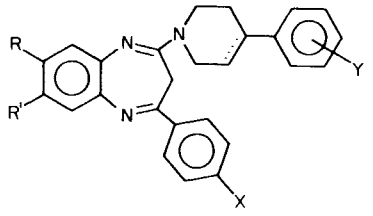
Biological Results. The compounds were evaluated in the four assays summarized in Table IV and described in detail in the Experimental Section.

Protection against amphetamine-induced stereotypy or lethality has been used as a criterion for evaluation of potential neuroleptic agents.[7,10] Test compounds were evaluated at doses of 5 and 50 mg/kg for their ability to inhibit *d*-amphetamine-induced lethality.[11] Compounds **5b** and **6i,k** were active at 5 mg/kg; **6a,e** and **7g** were active at both 5 and 50 mg/kg; and **6o** and **7h** were active at 50 mg/kg.

The compounds were tested at doses of 5 and 20 mg/kg for their effect on conditioned avoidance response (CAR) behavior in naive male rats. A significant decrease in avoidance responses was considered to indicate potential as a central nervous system depressant or tranquilizer (antipsychotic). Compounds **5c,e** were active at 20 mg/kg but not at 5 mg/kg. Conversely, **6e** was active at 5 mg/kg but not at 20 mg/kg; **6o** was active at both doses. For all of the compounds that showed some activity in the CAR assay, depression[12] was observed at similar doses. None of the 7 group of compounds was active at either dose.

Standard antipsychotic drugs increase brain dopamine (DA) turnover rates.[13] Compounds **5a,c,e**, **6a,m,o**, and **7i**

Table II. 2-(4-Arylpiperidino)-3*H*-4-aryl-1,5-benzodiazepines

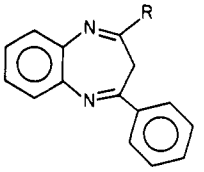


6

compd	R	R′	X	Y	····	% yield	mp, °C	recrystn solvent[a]	formula	analyses	λ max	ε max
6a						70	205–207.5	E	$C_{26}H_{25}N_3$	C, H, N	260 348	33700 4500
6b	Cl					48	134.5–136	D	$C_{26}H_{24}ClN_3$	C, H, N	263 358	40700 4800
6c	CH_3	CH_3				63	174.5–176.5	D	$C_{28}H_{29}N_3$	C, H, N	264.5 350	34000 5200
6d			CH_3			43	158–160	E	$C_{27}H_{27}N_3$	C, H, N	262.5 346	41400 5500
6e			F			44	173–175	E	$C_{26}H_{24}FN_3$	C, H, N	262 346	37500 4800
6f			OCH_3			53	158–160	E	$C_{27}H_{27}N_3O$	H, N; C[b]	267 340	40600 7600
6g			Cl			52	170–171	D	$C_{26}H_{24}ClN_3$	C, H, N	262 354	44700 5200
6h				*m*-CF_3		74	161.5–162.5	D	$C_{27}H_{24}F_3N_3$	C, H, N	260 347	36000 4600
6i				*p*-Cl		51	198.5–200	F	$C_{26}H_{24}N_3Cl$	C, H, N	260 346	40400 5400
6j				*p*-OCH_3		51	158.5–160	D	$C_{27}H_{27}N_3O$	C, H, N	260 346	36000 4500
6k				*p*-CF_3		79	157–158.5	D	$C_{27}H_{24}F_3N_3$	C, H, N	259 344	36000 4600
6l					=	68	180.5–182	F	$C_{26}H_{23}N_3$	C, H, N	258.5 345	45100 4600
6m				*p*-Cl	=	46	199–202	F	$C_{26}H_{22}ClN_3$	C, H, N	259 343	48600 4800
6n				*p*-CF_3	=	29	168–170	E	$C_{27}H_{22}F_3N_3$	C, H, N	260 342	46700 5000
6o				*p*-CH_3	=	24	208–211	E	$C_{27}H_{25}N_3$	C, H, N	258 342	48000 4700

[a] See footnote *b* of Table I. [b] C: calcd, 79.17; found, 78.71.

Table III. Other Benzodiazepineamidines



7

compd	R	% yield	mp, °C	recrystn solvent[a]	formula	analyses	λ max	ε max
7a	$N(Me)_2$	63	108-110.5	G	$C_{17}H_{17}N_3$	C, H, N	258 346	33300 4600
7b	N(Me)⌒OH	72	111-112.5	H	$C_{18}H_{19}N_3O$	C, H, N	258 346	35300 4800
7c	c-NC_4H_8	81	155-156	G	$C_{19}H_{19}N_3$	C, H, N	260 349	35000 4400
7d	c-NC_5H_{10}	64	115.5-118	A	$C_{20}H_{21}N_3$	C, H, N	260 348	34700 4600
7e	c-$N(CH_2CH_2)_2O$	70	150-152	G	$C_{19}H_{19}N_3O$	C, H, N	258 342	32100 4600
7f	c-$N(CH_2CH_2)_2N$-C_6H_5	52	172-172.5	D	$C_{25}H_{24}N_4$	C, H, N	256 346	43200 4700
7g	c-NC_5H_9-4-$CH_2C_6H_5$	29	97-99.5	B	$C_{27}H_{27}N_3$	C, H, N	260 346	34200 4600
7h	c-NC_5H_9-4-$CH(C_6H_5)_2$	58	170-172	D	$C_{33}H_{31}N_3$	C, H, N	260 346	35400 4500
7i	c-NC_5H_9-4-c-NC_5H_9	34	155-157	A	$C_{25}H_{30}N_4$	C, H, N	258.5 344	34900 4900

[a] See footnote *b* of Table I.

Table IV. Biological Evaluation of 5-7[h]

compd	blockade of *d*-amphetamine lethality[a]	CAR[b]	inhibn of DA-sensitive adenylyl cyclase[c]	DA turnover[d]	other observations[e]
clozapine	A (5)	A (5/20)	$IC_{50} = 1.4 \times 10^{-7}$ M	A	
5a	I	I	I	A	D, P (50)
5b	A (5), I (50)	I	*f*	I	
5c	I	I (5), A (20)	I	A	D (20), T (50)
5d	I	I	*f*	I	
5e	I	((5), A (20)	46% at 10^{-4} M	A	D (20, 50), P (50)
5f	I	I	*g*	*g*	
5g	I	I	*g*	*g*	
5h	I	I	*g*	*g*	
6a	A (5/50)	I	$IC_{50} = 4.5 \times 10^{-5}$ M	A	P (50)
6b	I	I	I	I	P (50)
6c	I	I	I	I	
6d	I	I	*f*	I	
6e	A (5/50)	A (5), I (20)	I	I	D (5)
6f	I	I	*f*	I	
6g	I	I	*f*	I	D, P (50)
6h	I	*f*	I	I	P (50)
6i	A (5), I (50)	I	*f*	I	P (30)
6j	I	*f*	I	I	
6k	A (5), I (50)	*f*	I	I	D (50)
6l	I	I	I	I	
6m	I	I	I	A	D, P (50)
6n	I	I	I	I	
6o	I (5), A (50)	A (5/20)	27% at 10^{-4} M	A	D (5, 20)
7a	I	I	*f*	I	P (50)
7b	I	I	*f*	I	
7c	I	I	*f*	I	
7d	I	I	*f*	I	
7e	I	I	*f*	I	
7f	I	I	*f*	I	
7g	A (5/50)	I	*f*	I	P (50)
7h	I (5), A (50)	I	*f*	I	P (50)
7i	I	I	I	A	

[a] Expressed as active (A) or inactive (I) at 5 and 50 mg/kg or inactive (I) at both doses. [b] Active (A) or inactive (I) at 5 and 20 mg/kg or inactive (I) at both doses. [c] Expressed as percent inhibition at the screening concentration of 10^{-4} M or as concentration necessary for 50% inhibition (IC_{50}). [d] Expressed as active (A) or inactive (I) relative to concurrently observed controls. [e] Observations made of the animals at expressed doses: D = depression, T = tremors, P = ptosis. [f] Not tested. [g] Not tested but inactive in an in vitro dopamine receptor assay. [h] See Tables I-III for structures.

were found to significantly increase the brain DA turnover rates in rats. Neuroleptics which increase DA turnover presynaptically through receptor blockade will inhibit the DA-stimulated adenylyl cyclase production of cAMP in

vitro.[14] It is possible to characterize potential neuroleptic agents by measuring their effect on DA turnover and their effects on striatal adenylyl cyclase. Of our compounds, only **6a** was found to inhibit the DA-stimulated enzyme activity by 50%, with an IC_{50} at 4.5×10^{-5} M. Two other compounds (**5e** and **6o**) showed minimal inhibition (46 and 27%, respectively) at the screening concentration of 10^{-4} M.

Discussion

The most significant aspects of the biological evaluation of these compounds were that there was decreased activity in the tests for potential neuroleptic activity relative to clozapine (or relative to other standard antipsychotic agents), and the activities that were observed lacked consistency. The biological testing, as we have reported the data, does not distinguish clozapine from the classical neuroleptics. However, because significant activity was not observed, such differentiation was not appropriate. From our extensive series of various 2-amino groups and aromatic substitutions, no structure–activity relationships (SAR) were apparent. Compound **6a** with a 4-phenyl substituent at the 2 position had activity in three of the four assays. Its IC_{50} in the dopamine-sensitive adenylyl cyclase assay of 4.5×10^{-5} M was encouraging but was considered minimal when compared with haloperidol and clozapine which had an IC_{50} of 2.3×10^{-6} and 1.4×10^{-7} M, respectively. This observation led to the synthesis of compounds **6b–o**. However, only **6o** showed indications of activity in all four assays and the activity in the DA assays was minimal.

Compounds of the **5** series with an *N*-methylpiperazino substituent at the 2 position were structurally more closely related to clozapine. Even though some of the desirable activities were observed (**5e**, for example, was active in three of the assays) none was considered to approach that of clozapine. Again inconsistencies of SAR were apparent: **5c** and **5e** had some CAR activity but **5h** was inactive. As a group, compounds of the miscellaneous category (**7a–i**) were void of the desired activities.

These results reflect that the structural modifications of **5–7**, relative to clozapine, resulted in a decrease in potential neuroleptic activity. It is quite apparent from our data that the 1,5-benzodiazepine portion of clozapine (**1**) is not responsible for its antipsychotic activity. Kukla[15] has recently reported on the synthesis of 1-amino- and 2-amino-5-phenyl-4,5-dihydro-3*H*-2-benzazepines as structural analogues of **1**. These compounds were also found to lack potential neuroleptic activities similar to **1**. Our data in conjunction with Kukla's may suggest that the tricyclic dibenzodiazepine portion of **1** is necessary for its psychotropic activities.

Experimental Section

General procedures are given for the preparation of the final compounds (**5–7**) for which data are given in Tables I–III. A typical example is given for the preparation of intermediates **3**, **4**, and **8**. The other intermediates were prepared by similar procedures. The secondary amines used for preparation of the amidines were either commercially available or were synthesized by known procedures.

NMR (Varian A-60D), IR (Beckman IR 12), and UV (Beckman DK2) spectra where applicable were consistent with all of the structures. Analytical results when indicated by symbols for the elements were within 0.4% of the theoretical values. Melting points were determined in open capillary tubes in a Mel-Temp apparatus or on a Fisher-Johns apparatus and are uncorrected.

Procedure Used for the Preparation of the Thiones 3. Preparation of 2,3-Dihydro-4-phenyl-7,8-dimethyl-1*H*-1,5-benzodiazepine-2-thione. *tert*-Amyl alcohol (109 g, 1.24 mol) was added dropwise to a stirred suspension of 52.0 g (1.24 mol) of 57% sodium hydride dispersion in 750 mL of dry benzene. The reaction mixture was stirred at reflux until the evolution of hydrogen ceased. The mixture was allowed to cool to ambient temperature and was filtered into a new round-bottom flask. After cooling in an ice bath a solution of 75.0 g (0.625 mol) of acetophenone and 94.0 g (1.24 mol) of carbon disulfide was added dropwise with stirring. The mixture was left standing at ambient temperature overnight; then water was added and the benzene layer was extracted with several portions of water. The combined aqueous portions were washed with ether and made acidic by the addition of 10% H_2SO_4. The solid was extracted into ether, washed with water, and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave a solid that was washed with hexane and dried, yielding 69.6 g (56.8%) of benzoyldithioacetic acid as an orange powder, mp 59–60 °C (lit.[6] mp 63 °C). The dithioacetic acids **8** prepared in this manner were used without further purification.

A mixture of 6.8 g (50 mmol) of 4,5-dimethyl-*o*-phenylenediamine and 9.8 g (50 mmol) of benzoyldithioacetic acid in 250 mL of xylene was stirred at 110–130 °C for 2.5 h and then allowed to cool. The crystals were collected, washed with hexane, and dried, yielding 11.6 g (83%) of beige crystals of 2,3-dihydro-4-phenyl-7,8-dimethyl-1*H*-1,5-benzodiazepine-2-thione. Recrystallization of 3.50 g from EtOAc gave 2.53 g of off-white crystals, mp 215.5–218 °C. Anal. ($C_{17}H_{16}N_2S$) C, H, N, S. The crude products were generally satisfactory for further reaction.

General Procedures for the Preparation of Methylthioimino Ethers 4. To a stirred suspension of 21 mmol of the thiolactam **3** in 400 mL of anhydrous benzene was added 30 mmol of 57% sodium hydride dispersion. The mixture was stirred at reflux for 2 h; then 30 mmol of methyl iodide was added and reflux was continued overnight. The mixture was filtered and the filtrate was concentrated under reduced pressure leaving an oil that was used without further purification. (This product also contained the mineral oil from the sodium hydride dispersion.)

The products were characterized by their NMR and UV spectra: NMR (for the unsubstituted compound, 2-methylthio-4-phenyl-3*H*-1,5-benzodiazepine) ($CDCl_3$) 2.47 (3 H, s) and 3.49 ppm (2 H, s); UV λ max (MeOH) 253.5 nm (ε 28 500).

General Procedures for the Preparation of the Amidines 5–7. (A) Preparation of 2-(4-Methylpiperazino)-4-aryl-3*H*-1,5-benzodiazepines (5). A solution of 15 mmol of the methylthioimino ether **4**, 7 mL of *N*-methylpiperazine, and 1 mL of glacial acetic acid in 45 mL of chloroform was stirred under reflux overnight. The solvent was removed under reduced pressure and the residue was partitioned between benzene and water. The benzene portion was washed once with water and then was extracted with eight 25-mL portions of 10% aqueous acetic acid. The combined extracts were washed once with benzene and then the acidic solution was made alkaline by the addition of concentrated ammonium hydroxide. The resultant mixture was extracted with benzene; the extracts were washed with water and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave a crude product which was recrystallized as indicated in Table I.

(B) Preparation of 2-(4-Arylpiperidino)-4-aryl-3*H*-1,5-benzodiazepines 6. A mixture of 20 mmol of the appropriate methylthioimino ether, 15–40 mmol (ratios of reactants depended on their relative availability) of a 4-arylpiperidine or 4-aryltetrahydropyridine, and 10 drops of glacial acetic acid in 45 mL of toluene was stirred under reflux overnight. The reaction solution was diluted with 50 mL of benzene, washed successively with five 50-mL portions of 10% aqueous HOAc, two 25-mL portions of 2.5% NaOH, and water, and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave a residue that was recrystallized as indicated in Table II.

Other solvents could be used for these reactions (including dimethoxyethane, benzene, chloroform, and 2-butanone). Toluene was generally preferred as it provided faster reaction.

(C) Preparation of the Compounds (7) of Table III. The miscellaneous group of compounds (**7**) were prepared via procedures similar to the above. With the exception of **7a** which was carried out in chloroform at ambient temperature, all other reactions were achieved in refluxing chloroform. The reactions were worked up according to the procedure used for **5** if the products were soluble in 10% aqueous HOAc or according to the

procedure used for **6** if the products were not soluble in aqueous acetic acid. Yields and properties of these compounds are given in Table III.

Biological Evaluation. The compounds were evaluated in the four assays detailed below.

a. Blockade of *d*-Amphetamine-Induced Lethality. At least 0.5 h before drug administration, groups of ten mice were brought into an experimental cubicle. Room temperature was maintained at approximately 22 °C. Thirty minutes following ip injection of vehicle or doses of the test drug, *d*-amphetamine (115 mg/kg ip) was given and each animal was placed in an individual observation cage. This dose of *d*-amphetamine induced a rapid onset of 80–100% lethality.[11] Thirty minutes after *d*-amphetamine administration, the number of mice surviving at each test drug dosage level was recorded. The number of survivals was compared with survivals for the concurrent controls.

Test compounds were evaluated at doses of 5 and 50 mg/kg. A compound was rated active if there was a 50% or greater survival as compared to controls. Clinically active antipsychotic agents (including chlorpromazine, haloperidol, fluphenazine, clozapine, and butaclamol) are active in this assay under these conditions.

b. Conditioned Avoidance Response (CAR). The apparatus consisted of a shuttle box divided into two compartments and enclosed in a sound attenuating chamber. The floor of the shuttle box was an electrifiable grid. Following intraperitoneal injection of the vehicle or the drug, the rat was placed into the shuttle cage and was allowed to acclimate for approximately 1 min. A 5-s conditioned stimulus, consisting of a tone and light, preceded a 0.2-mA footshock delivered to the grid floor of the cage. The shock was automatically terminated after 30 s if the rat failed to respond. A shuttle response during the conditioned stimulus prevents the onset of the shock and was scored as an avoidance response. If no escape response was made an escape failure was scored. Each conditioned stimulus presentation was separated by a 15-s interval and a response during this interval was scored as an intertrial interval response and results in the onset of the shock and the conditioned stimulus until the rat returned to the other side. Each rat was presented with 100 trials (i.e., 100 conditioned stimulus presentations) which took approximately 30 min. Twelve rats per dose were tested and each dose group was compared to the control group run concurrently by means of a Student's *t* test ($p < 0.05$, two tailed). Most drugs were evaluated 30 min postinjection but intervals of 1–90 min can be used.

Compounds which did not alter avoidance response or intertrial interval responses were considered inactive. Compounds which significantly decreased avoidance responses were considered as central nervous system depressants or tranquilizers (neuroleptics). Test compounds were assayed at 5 and 20 mg/kg. Drugs currently in clinical use as tranquilizers showed activity in this test.

c. Brain Dopamine Turnover. Rats were given a single intraperitoneal injection of saline or test compound 30 min after a 200 mg/kg intraperitoneal dose of α-methyl-*p*-tyrosine, a known tyrosine hydroxylase inhibitor. At 1- and 2-h time intervals following intraperitoneal administration of 25 or 30 mg/kg of test compound, the rats (six per time point) were sacrificed; their brains were removed and assayed[16] for dopamine content. Basal dopamine levels were determined from rats sacrificed immediately after a saline injection. The time necessary for disappearance of 50% of this initial dopamine concentration ($t_{1/2}$) was determined by plotting dopamine levels vs. time at 0, 1, and 2 h following test compound administration. Compounds that significantly increased brain dopamine turnover were rated active ($p < 0.05$ as rated by a Student's *t* test). Standard antipsychotic drugs that were tested and active in this assay include pimozide, chlorpromazine, haloperidol, thioridazine, and clozapine.

d. Inhibition of Dopamine-Sensitive Adenylyl Cyclase Activity in Vitro. Male rats (Charles River Laboratories) weighing 225–250 g were sacrificed by cervical dislocation and the entire brain was quickly removed and chilled on crushed ice. Corpora striata from two or three animals were removed using a dissection procedure outlined by Kebabian and co-workers.[17] The pooled striatal tissue was then homogenized in 50 vol of ice-cold 20 mM Tris HCl (pH 7.4) containing 2 mM EGTA to yield a crude adenylyl cyclase suspension. The standard incubation mixture (final volume 1.0 mL) contained (in millimoles per liter) Tris HCl (pH 7.4), 8.0; ATP, 1.5; $MgSO_4$, 2.0; theophylline, 10; EGTA, 0.2; 100 μL of homogenate suspension; plus test substances. Dopamine was dissolved in 0.01 N HCl and 10-μL aliquots were added to the incubation mixture. Whenever possible test compounds were solubilized in 0.01 N HCl. Nonaqueous soluble drugs were dissolved in absolute ethanol using heat and/or a few drops of 4 N HCl if necessary. In all cases, drugs were added as 10-μL aliquots and 10 μL of the appropriate vehicle was added to control samples. Absolute ethanol did not affect basal or stimulated cAMP levels at the volumes used.

Reactions were initiated by the addition of homogenate and samples were incubated for 5 min with constant shaking in a 30 °C water bath. Reactions were terminated by placing samples in boiling water for 3 min. Following this deproteinization samples were centrifuged to remove insoluble materials and the supernatant was assayed for cAMP content using the competitive binding procedure described by Brown et al.[18] Known antipsychotic agents inhibit dopamine stimulation of adenylyl cyclase activity in a concentration range of 10^{-8}–10^{-5} M.[14b,d]

Acknowledgment. The authors express their appreciation to Mr. James Bloss for biological data, to Mr. Aristides Damascus for spectral data, to Mr. Emmanuel Zielinski for microanalytical data, to Drs. Michael Kukla, Fred Hershenson, and George Clay for helpful discussions, and to Mrs. Rosemary Whitmore for help in preparation of the manuscript.

References and Notes

(1) J. Schmutz, *Arzneim.-Forsch.*, **25**, 712 (1965).
(2) J. Angst, D. Bente, P. Berner, H. Heimann, H. Helmchen, and H. Hippus, *Pharmakopyschiatr./Neuro-Psychopharmakol.*, **4**, 201 (1971).
(3) L. H. Sternbach in "The Benzodiazepines", S. Garattini, E. Mussini, and L. O. Randall, Ed., Raven Press, New York, N.Y., 1973, p 1 ff.
(4) (a) S. H. Snyder, *Arch. Gen. Psychiat.*, **27**, 169 (1972); (b) J. Stevens, *ibid.*, **29**, 177 (1973); (c) for a review see H. Y. Meltzer and S. M. Stahl, *Schizophr. Bull.*, **2**, 19 (1976).
(5) H. R. Burki, E. Eichenberger, A. C. Sayers, and T. G. White, *Pharmakopsychiatr./Neuro-Psychopharmakol.*, **8**, 115 (1975).
(6) A. S. Horn and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2325 (1971).
(7) P. A. J. Janssen, C. J. E. Niemegeers, and K. H. L. Schellekens, *Arzneim.-Forsch.*, **15**, 104 (1965).
(8) A. Thuillier and J. Vialle, *Bull. Soc. Chim. Fr.*, 1398 (1959).
(9) D. Nardi, A. Tajana, and S. Rossi, *J. Heterocycl. Chem.*, **10**, 815 (1973).
(10) C. D. Proctor, J. L. Potts, R. O. Lundy, and E. J. Greenfield, *Arch. Int. Pharmacodyn. Ther.*, **163**, 79 (1966).
(11) (a) J. L. Bloss, private communication, Searle Laboratories; (b) test procedure described by J. L. Bloss and G. H. Singer, *Psychopharmacology*, in press.
(12) This assay does not distinguish a CNS depressant from a tranquilizer. The activity observed for our compounds does not correlate with activity in the other antipsychotic assays, except for compound **6o**, perhaps indicating that some sedative or other effect is responsible for the CAR activity.
(13) (a) H. Nyback, Z. Borzecki, and G. Sedvall, *Eur. J. Pharmacol.*, **4**, 395 (1968); (b) S. Mathysse, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 200 (1972).
(14) (a) R. J. Miller and L. L. Iverson, *J. Pharm. Pharmacol.*, **26**, 142 (1974); (b) Y. C. Clement-Cormier, J. W. Kebabian, G. L. Petzold, and P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1113 (1974); (c) M. Karobath and H. Leitich, *ibid.*, **71**, 2915 (1974); (d) L. L. Iverson, *Science*, **188**, 1084 (1975).
(15) M. J. Kukla, *J. Heterocycl. Chem.*, **14**, 933 (1977).
(16) R. Laverty and K. M. Taylor, *Anal. Biochem.*, **22**, 269 (1968).
(17) J. W. Kebabian, G. L. Petzold, and P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2145 (1972).
(18) B. L. Brown, J. D. M. Albano, R. P. Ekins, and A. M. Sgherzi, *Biochem. J.*, **121**, 561 (1971).