Neuroleptic Activity in 5-Aryltetrahydro-γ-carbolines

Charles A. Harbert,* Jacob J. Plattner, Willard M. Welch,

Department of Medicinal Chemistry

Albert Weissman, and B. Kenneth Koe

Department of Pharmacology, Pfizer Central Research Laboratories, Groton, Connecticut 06340. *Received November 8, 1979*

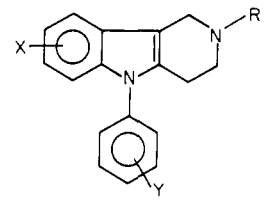
A series of 5-aryltetrahydro-γ-carbolines was prepared by a novel N-arylation procedure and tested for neuroleptic activity in a rat antiamphetamine model. The systematic exploration of structural parameters leading to 8-fluoro-5-(4-fluorophenyl)-2-[4-hydroxy-4-(4-fluorophenyl)butyl]-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (CP-36,584, flutroline), a potent and long-acting neuroleptic compound, is described. These semirigid compounds provide a new, structurally distinct series with which to probe the conformational requirements for potent activity at the dopamine receptor.

Since the discovery of chlorpromazine as an effective antipsychotic agent, large numbers of potential neuroleptics have been synthesized. Despite this intense research activity, there are still relatively few structurally distinct classes of antipsychotic drugs which have confirmed clinical activity.[1] One of the largest classes by far is the linear tricyclic series characterized by 6,6,6 or 6,7,6 fused ring systems with an amine-containing side chain originating from the central ring. Examples of the 6,6,6 system are the phenothiazines, which includes chlorpromazine, and the thioxanthenes, of which thiothixene is an example. Examples of the 6,7,6 system include the dibenzodiazepines, such as clozapine, and the dibenzoxazepines, such as loxapine.

The butyrophenones, exemplified by haloperidol, represented the first major departure from the tricyclic skeleton. Related chemical classes are the diphenylbutylamines, such as pimozide, and the isosteric series of aminoethylbenzamides, of which sulpiride is the prototype. The dihydroindoles, from which molindone is derived, are still another class distinct from the tricyclics. The benzocycloheptapyridoisoquinolines represented by (+)-butaclamol are another structurally distinct series of new experimental agents distinguished by their conformational rigidity. A very useful classification of neuroleptics, by atom sequencing from the aromatic ring to the basic nitrogen, has appeared elsewhere.[2]

All established antipsychotic agents regardless of structure are thought to act by blockade of central dopamine receptors,[3,4] and at least two conformations have been proposed to account for this interaction. The first is an extended phenethylamine conformation, based on the observation that the solid-state conformation of chlorpromazine overlaps nearly perfectly with the extended form of dopamine.[5] The marked neuroleptic potency of butaclamol, which contains a conformationally restricted phenethylamine moiety,[6] lends support to the dopamine overlap hypothesis. A second conformation, which was proposed by Janssen,[2] consists of an "S-shaped" arrangement of the four-atom sequence that links the aromatic ring to the basic nitrogen in many open-chain neuroleptics. The potent neuroleptic activity of a series of piperidylidenethioxanthenes[7] that incorporate a semirigid "S-shaped" conformation, but are incapable of adopting the conformation of solid-state chlorpromazine, is consistent with the latter hypothesis.

In earlier communications,[8,9] we have reported the discovery of marked neuroleptic activity in a series of 5-aryltetrahydro-γ-carbolines (I), which is structurally distinct from any of the above classes of antipsychotic agents and which contains both an extended phenethylamine and an S-shaped four-atom sequence in a conformationally restricted structure. Based on the high in vitro and in vivo potency of the 5-aryltetrahydro-γ-carbolines, we have proposed[9] that the interaction of dopamine agonists and antagonists with the central dopamine receptor may be achieved by their ability to position an aromatic ring and a basic nitrogen atom in a spatial relationship approximated by the carboline nucleus. We further proposed[9] that the open-chain diphenylbutylpiperidine and butyrophenone neuroleptics assume this relative positioning of aromatic ring and basic nitrogen at the dopamine receptor and thereby adopt an S shape. As such, the conformation of the carboline nucleus appears to reconcile the previously disparate proposals—S shape and extended phenethylamine—for interaction at the dopamine receptor. This paper describes in detail the synthesis of this series of compounds by a useful N-arylation procedure and structure–activity relationships within this series.



I

The 5-aryltetrahydro-γ-carbolines, unlike their 5-dearyl counterparts, have not been extensively examined. 2-Methyl-5-phenyltetrahydro-γ-carboline was prepared as one of a series of 5-substituted derivatives tested for antihistaminic activity.[10] A related series of 5-aryltetra-

(1) C. L. Zirkle and C. Kaiser in "Medicinal Chemistry", 3rd ed, Part II, A. Burger, Ed., Wiley-Interscience, New York, 1970, pp 1410–1469.
(2) P. A. J. Janssen in "Modern Problems in Pharmacopsychiatry, The Neuroleptics", Vol. 5, D. P. Bobon, P. A. J. Janssen, and J. Bobon, Eds., S. Karger, Basel, 1970, p 33.
(3) A. Carlsson and M. Lindquist, *Acta Pharmacol. Toxicol.*, **20**, 140 (1963).
(4) I. Creese, D. R. Burt, and S. H. Snyder, *Science*, **192**, 481 (1976).
(5) A. S. Horn and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2325 (1971).
(6) L. G. Humber, F. T. Bruderlein, and K. Voith, *Mol. Pharmacol.*, **11**, 833 (1975).
(7) C. Kaiser, R. J. Warren, and C. L. Zirkle, *J. Med. Chem.*, **17**, 131 (1974).
(8) B. K. Koe, A. Weissman, J. J. Plattner, C. A. Harbert, and W. M. Welch, *Pharmacologist*, **21**, 180 (1979).
(9) C. A. Harbert, J. J. Plattner, W. M. Welch, A. Weissman, and B. K. Koe, *Mol. Pharmacol.*, **17**, 38 (1980).
(10) U. Horlein, *Chem. Ber.*, **87**, 463 (1954); British Patents 752688 (1954), 721771 (1952), 733123 (1953).

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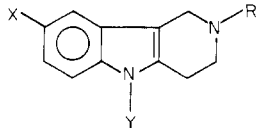
Table I. 2-Carbethoxy-5-aryl-1,2,3,4-tetrahydro-γ-carbolines



no.	X	Y	R	method[a]	% yield	mp, °C	formula[b]
1	Cl	H	$COOC_2H_5$	A	62	188-190	$C_{14}H_{15}N_2O_2Cl$
2	Br	H	$COOC_2H_5$	A	63	192-193	$C_{14}H_{15}N_2O_2Br$
3	F	H	$COOC_2H_5$	A	69	169-170	$C_{14}H_{15}N_2O_2F$
4	H	H	$COOC_2H_5$	A	60	121-123	$C_{14}H_{16}N_2O_2$
5	CH_3	H	$COOC_2H_5$	A	72	160-163	$C_{15}H_{18}N_2O_2$
6	OCH_3	H	$COOC_2H_5$	A	63	159-160	$C_{15}H_{18}N_2O_3$
7	Cl	C_6H_5	$COOC_2H_5$	B	65	113-114	$C_{20}H_{19}N_2O_2Cl$
8	Br	C_6H_5	$COOC_2H_5$	B	46	133-135	$C_{20}H_{19}N_2O_2Br$
9	F	C_6H_5	$COOC_2H_5$	B	61	62-65	$C_{20}H_{19}N_2O_2F$
10	H	C_6H_5	$COOC_2H_5$	B	40	oil	$C_{20}H_{20}N_2O_2$
11	CH_3	C_6H_5	$COOC_2H_5$	B	62	105-108	$C_{21}H_{22}N_2O_2$
12	OCH_3	C_6H_5	$COOC_2H_5$	B	47	115-117	$C_{21}H_{22}N_2O_3$
13	Cl	4-FC_6H_4	$COOC_2H_5$	B	69	87-89	$C_{20}H_{18}N_2O_2ClF$
14	F	4-FC_6H_4	$COOC_2H_5$	B	78	121-123	$C_{20}H_{18}N_2O_2F_2$
15	OCH_3	4-FC_6H_4	$COOC_2H_5$	B	47	107-110	$C_{21}H_{21}N_2O_3F$
16	F	4-FC_6H_4	$COCH_3$	C	97	150-152	$C_{19}H_{16}N_2OF_2$
17	F	4-FC_6H_4	COC_2H_5	C	79	133-134	$C_{20}H_{18}N_2OF_2$
18	F	4-FC_6H_4	$COCH_2C(CH_3)_3$	C	77	133-135	$C_{23}H_{24}N_2OF_2$
19	F	4-FC_6H_4	COC_6H_5	C	84	141-144	$C_{24}H_{18}N_2OF_2$
20	Cl	C_6H_5	H	D	75	276-278	$C_{17}H_{15}N_2Cl\cdot HCl$
21	Br	C_6H_5	H	D	25	280-282	$C_{17}H_{15}N_2Br\cdot HCl$
22	F	C_6H_5	H	D	68	210-212	$C_{17}H_{15}N_2F\cdot HCl$
23	H	C_6H_5	H	D	45	271-272	$C_{17}H_{16}N_2\cdot HCl$
24	CH_3	C_6H_5	H	D	70	288-289	$C_{18}H_{18}N_2\cdot HCl$
25	Cl	4-FC_6H_4	H	D	89	269-271	$C_{17}H_{14}N_2ClF\cdot HCl$
26	F	4-FC_6H_4	H	D	41	127-128	$C_{17}H_{14}N_2F_2$

[a] See Experimental Section for general preparative procedures. [b] All compounds were analyzed for C, H, and N. Except where noted, values obtained agreed with calculated values within ±0.4%.

hydro-γ-carbolines has been reported elsewhere to lack significant CNS depressant activity.[11]

Chemistry. Prior to this work, the only known 5-aryl-1,2,3,4-tetrahydro-γ-carbolines were simple phenyl-substituted derivatives.[10,11] Although experimental details are lacking, these appear to have been synthesized from 1,1-diphenylhydrazine by the Fischer synthesis. For the purposes of this work, a route amenable to considerable variation was desired in order to facilitate introduction of a diversity of substituents in both the indole and 5-aryl rings. In addition, consideration was given to the preparation of intermediates from which analogues at position 2 could be conveniently synthesized. The general unavailability of substituted 1,1-diarylhydrazines, the problems associated with stability of such compounds (in particular, those with electron-withdrawing substituents), and the difficulty of obtaining single products from unsymmetrically substituted hydrazines precluded the Fischer reaction as a synthetic method leading directly to the 5-aryl-substituted carbolines.

The Ullman reaction has been used classically for the synthesis of diphenylamines from acylanilines and substituted aromatic halides.[12] Since the basicity of *N*-acylanilines and of indole and its derivatives is greatly reduced by electron delocalization, it seemed reasonable to assume that indole derivatives would be acceptable substrates for the Ullman procedure. With certain modifications, this proved to be the case.

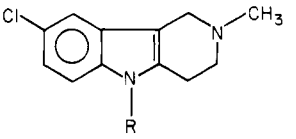
The substrates chosen for the present experiments were the N^2-carbethoxy-1,2,3,4-tetrahydro-γ-carbolines **1–6** (Table I), readily available by standard Fischer synthesis from the commercially available substituted phenylhydrazines and *N*-carbethoxy-4-piperidinone. The *N*-carbethoxy group served several purposes in this synthesis: The Fischer reaction proceeds in better yield when the nitrogen atom of 4-piperidinone is nonbasic, possibly due to suppression of retro-Michael reactions leading to polymerization in the acidic media; the *N*-carbethoxy group serves as a precursor for 2-methyl derivatives by reduction or to 2-alkyl derivatives by hydrolysis and alkylation; and the modified Ullman reaction described below gives substantially higher yields when a 2-acyl substituent is present rather than a 2-alkyl substituent.

Treatment of compounds **1–6** with several equivalents of a suitably substituted aromatic halide in the presence of cuprous bromide and inorganic base at elevated temperature gave reasonable yields (40–76%) of the 5-phenyl (compounds **7–12**) and 5-(4-fluorophenyl) derivatives (compounds **13–15**). The choice of solvent for this reaction was critical in that much better results were obtained when higher boiling (bp 202 °C) anhydrous *N*-methyl-2-pyrrolidinone was used instead of the more commonly used solvents nitrobenzene or dimethyl formamide. Other azoles, such as indole itself, carbazole and phenothiazine were also readily N-arylated by this procedure.[13]

The desired 2-methyl-5-aryltetrahydro-γ-carbolines were obtained by an aluminum hydride reduction of the corresponding N^2-carbethoxy-substituted derivatives. Use of lithium aluminum hydride as reducing agent was less satisfactory due to formation of the N-demethylated derivatives as side products. To prepare the series of 2-alkyl- and 2-(arylalkyl)-5-aryl derivatives, the carbethoxy group at the 2 position was removed hydrolytically with ethanolic

(11) R. G. W. Spickett, *J. Med. Chem.*, 9, 436 (1966).
(12) M. A. Khan, *Rec. Chem. Prog.*, 31, 43 (1970).
(13) Unpublished results.

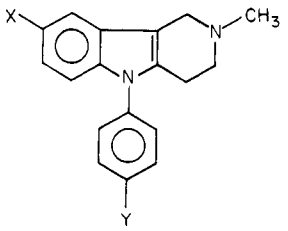
Table II. Effect of 5-Aryl Substitution on Neuroleptic Activity



no.	R	method[a]	mp, °C	formula[b]	antagonism of amphetamine (rat): ED_{50}, mg/kg ip[c] 1 h	5 h	24 h
27	H	E	196-198	$C_{12}H_{13}N_2Cl$	32-56	NT[d]	NT
28	CH_3	E	286-287	$C_{13}H_{15}N_2Cl \cdot HCl \cdot 0.33H_2O$	32-56	NT	NT
29	C_6H_5	E	281-283	$C_{18}H_{17}N_2Cl \cdot HCl$	3.2-10	10-32	>32
30	4-FC_6H_4	E	286-288	$C_{18}H_{16}N_2ClF \cdot HCl$	1.0-3.2	3.2-10	>10
chlorpromazine					3.2-10	>17.8	>56
thiothixene					0.32-1.0	0.32-1.0	>17.8

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] Entries are ranges within which fall the ED_{50} values for blocking hyperactivity and stereotypy induced by amphetamine. Details are given under Experimental Section. [d] Not tested.

Table III. Effect of Variation of the 8 Substituent on Neuroleptic Activity



no.	X	Y	method[a]	mp, °C	formula[b]	antagonism of amphetamine (rat): ED_{50}, mg/kg ip[c] 1 h	5 h	24 h
31	OCH_3	H	E	265-266	$C_{19}H_{20}N_2O \cdot HCl$	>32	>32	NT
32	CH_3	H	E	128-130	$C_{19}H_{20}N_2$	10-32	>32	>32
33	H	H	E	247-250	$C_{18}H_{18}N_2 \cdot HCl$	10-32	NT	>32
34	Br	H	E	279-281	$C_{18}H_{17}N_2Br \cdot HCl$	3.2-10	NT	NT
29	Cl	H	E			3.2-10	10-32	>32
35	F	H	E	250-252	$C_{18}H_{17}N_2F \cdot HCl \cdot 0.5H_2O$	3.2-10	10-32	>32
30	Cl	F	E			1.0-3.2	3.2-10	>10
36	OCH_3	F	E	262-265	$C_{19}H_{19}N_2OF \cdot HCl$	>32	>32	NT
37	F	F	E	295-297	$C_{18}H_{16}N_2F_2 \cdot HCl$	1.0-3.2	3.2-10	>10

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table II.

potassium hydroxide, and the resulting secondary amines were then directly alkylated, or acylated and then reduced, to give the desired analogues (Scheme I).

Esters of **52** (compounds **80–84**, Table VII) were prepared by standard acylation conditions as described under Experimental Section.

Pharmacology and Discussion. Neuroleptic activity of the compounds listed in Tables II–VI was assessed by their ability to antagonize the stereotypy induced in rats by *d*-amphetamine sulfate (5 mg/kg, ip) as described under Experimental Section. Amphetamine was given 1, 5, 24, and, occasionally, 48 and 72 h after drug administration to assess duration of action of agents discussed below. Although intraperitoneal (ip) testing results are tabulated, all compounds were active orally at one to three times the ip doses. The duration of action of compounds listed in Table VII was determined by their ability to antagonize the emetic effect of iv apomorphine in dogs as described by Weissman.[14]

Physical properties of intermediate carbethoxy and acyl derivatives of the basic 5-aryltetrahydro-γ-carboline nucleus and of the derived secondary amines are presented in Table I. None of the compounds in which the nitrogen atom at position 2 is nonbasic by virtue of its amide character were active in the neuroleptic models. Furthermore, none of the secondary amines **20–26** presented in Table I displayed activity at the doses tested (up to 32 mg/kg ip). These results were not unexpected; with the possible exception of amoxapine,[15] no neuroleptic agent lacking a basic nitrogen atom has been reported nor has any secondary amine yet been shown to display antipsychotic activity.

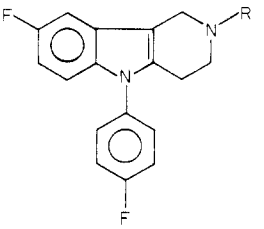
Table II illustrates the effect of aryl substitution at the 5 position on potency and duration of action in a simple 8-chloro-2-methyl series. The known compounds **27** and **28**,[16] with hydrogen and methyl substitution at N-5, respectively, exhibited antiamphetamine activity only at very high doses; phenyl substitution at this position (compound **29**) greatly enhanced potency, which was further increased in the 4-fluorophenyl derivative **30**. The activity of **30** in the antiamphetamine test exceeded that of chlorpromazine and was approximately one-third that of thiothixene. Of particular note also was the fact that aryl substitution at N-5 served not only to increase potency but also appeared

(14) A. Weissman, *Psychopharmacologia*, **12**, 142 (1968).

(15) E. N. Greenblatt, A. S. Lippa, and A. C. Osterberg, *Arch. Int. Pharmacodyn. Ther.*, **233**, 107 (1978).

(16) C. J. Cuttanach, A. Cohen, and B. Heath-Brown, *J. Chem. Soc.*, 1236 (1968).

Table IV. Variation of the 2 Substituent in the 8-Fluoro-5-(4-fluorophenyl) Series



no.	R	method[a]	mp, °C	formula[b]	antagonism of amphetamine (rat): ED_{50}, mg/kg ip[c] 1 h	5 h	24 h
14	$COOC_2H_5$	B			>32	>32	>32
26	H	D			10-32	NT	NT
37	CH_3	E			1.0-3.2	>10	>10
38	C_2H_5	E	250-253	$C_{19}H_{18}N_2F_2 \cdot HCl$	3.2-10	>10	>10
39	C_3H_7	E	276 (dec)	$C_{20}H_{20}N_2F_2 \cdot HCl$	3.2-10	>10	NT
40	$CH_2CH_2C(CH_3)_3$	E	274-276	$C_{23}H_{26}N_2F_2 \cdot HCl$	>10	>10	>10
41	$CH_2C_6H_5$	E	288-291	$C_{24}H_{20}N_2F_2 \cdot HCl \cdot 0.33H_2O$	3.2-10	>10	>10
42	$CH_2CH_2C_6H_5$	F	275-276	$C_{25}H_{22}N_2F_2 \cdot HCl \cdot 0.33H_2O$	10-32	>32	NT
43	$CH_2CH_2CH{=}CH(4\text{-}FC_6H_4)$	G	258-259	$C_{27}H_{23}N_2F_3 \cdot HCl$	3.2-10	1.0-3.2	>3.2
44	$CH_2CH_2CH_2CH_2(4\text{-}FC_6H_4)$	H	238-240	$C_{27}H_{25}N_2F_2 \cdot HCl \cdot 0.5H_2O$	0.32-1.0	0.32-1.0	3.2-10
45	$CH_2CH_2CH{=}C(4\text{-}FC_6H_4)_2$	G	140-142	$C_{33}H_{26}N_2F_4$	>32	>32	>32
46	$CH_2CH_2CH_2CH(4\text{-}FC_6H_4)_2$	H	224-226	$C_{33}H_{28}N_2F_2 \cdot HCl \cdot 0.25H_2O$	>32	>32	>32
47	$CH_2CH_2CO(4\text{-}FC_6H_4)$	F	195-198	$C_{26}H_{21}ON_2F_3 \cdot HCl$	>32	>32	>32
48	$CH_2CH_2CHOH(4\text{-}FC_6H_4)$	I	237-239	$C_{26}H_{23}ON_2F_3 \cdot HCl$	3.2-10	>10	>10
49	$CH_2CH_2CH_2CO(4\text{-}FC_6H_4)$	F	237-238	$C_{27}H_{23}ON_2F_3 \cdot HCl$	1.0-3.2	1.0-3.2	1.0-3.2
50	$CH_2CH_2CH_2CHOH(4\text{-}FC_6H_4)$	I	249-250	$C_{27}H_{25}ON_2F_3 \cdot HCl \cdot 0.5H_2O$	0.1-0.32	0.1-0.32	0.32-1.0
51	$(CH_2)_4CO(4\text{-}FC_6H_4)$	F	242-244	$C_{28}H_{25}ON_2F_3 \cdot HCl$	>32	>32	>32
52	$(CH_2)_4CHOH(4\text{-}FC_6H_4)$	I	207-208	$C_{28}H_{27}ON_2F_3 \cdot HCl$	1.0-3.2	1.0-3.2	>3.2
II[d]					1.0-3.2	3.2-10	>32
III[d]					10-32	>32	NT
IV[d]					3.2-10	>10	>10
V[d]					10-32	10-32	>32

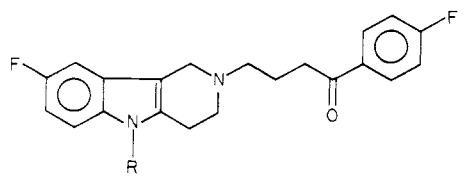
[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table II. [d] See text for structures.

to prolong activity in the antiamphetamine procedure, as indicated by the activity 5 h following drug administration.

Variation of the substituent at the 8 position in 2-methyl-5-phenyl and 5-(4-fluorophenyl) derivatives (Table III) revealed that electron-withdrawing (halogen) substituents in this position were superior to the corresponding 8-hydrogen, 8-methyl, and 8-methoxy substituents. Based on these results, the 8-fluoro-5-(4-fluorophenyl)-substituted nucleus was selected for the development of SAR at position 2.

Results presented in Table IV show that within the series of simple homologous compounds **37–40** increased lipophilicity and steric bulk of alkyl substituents at the 2 position led to slightly diminished activity in the antiamphetamine procedure. The secondary amine **26**, with hydrogen substitution at position 2, was again less potent than simple alkyl-substituted derivatives. Substitution of simple aralkyl groups, e.g., benzyl (**41**) and phenethyl (**42**), also reduced potency relative to the methyl analogue (**37**).

It has previously been reported that the butyrophenone-substituted tetrahydro-γ-carboline II possesses



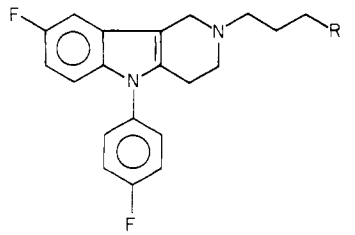
II, R = H
III, R = CH_3

analgetic and neuroleptic properties[17] in animals. In our hands, II exhibited antiamphetamine activity in the dosage range of 1.0–3.2 mg/kg, although the effect of this agent and its methyl congener III deteriorated at 5 and 24 h after treatment (Table IV). Having demonstrated that substitution at position 5 enhanced both antiamphetamine potency as well as duration of action of such compounds, preparation of analogues of **26** bearing various butyrophenone-derived substituents at position 2 was undertaken. As predicted, compounds with markedly increased potency and duration of action relative to II and III were obtained.

Initial investigation of this facet of the SAR at position 2 was directed toward preparation of **49**, the 5-(4-fluorophenyl) analogue of II, and of several analogues of **49** with varying side-chain lengths. In Table IV, it will be noted that **49** not only possessed impressive activity vs. amphetamine in the rat, but that this activity persisted for a period of at least 24 h. The next lower and higher homologues, **47** and **51**, respectively, were considerably less active, demonstrating a strict dependence upon side-chain length. Interestingly, reduction of **49** to the *dl* mixture of benzylic alcohols **50** led to a surprising tenfold increase in potency. Compound **50** is, in fact, among the most potent and long-lasting orally active neuroleptic agents in rodents reported thus far, with the effect following oral or ip dosing persisting for over 24 h. Upon further investigation, the

(17) E. T. Kimura, P. W. Dodge, P. R. Young, and R. P. Johnson, *Arch. Int. Pharmacodyn. Ther.*, **190**, 124 (1971).

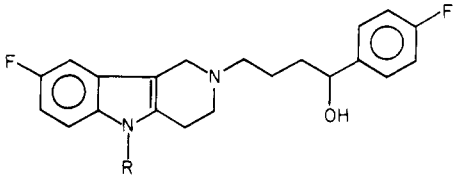
Table V. Variation of the Side-Chain Aromatic Ring Substitution Pattern



no.	R	method[a]	mp, °C	formula[b]	antagonism of amphetamine (rat): ED_{50}, mg/kg ip[c] 1 h	5 h	24 h
49	CO(4-FC_6H_4)	F			1.0-3.2	1.0-3.2	1.0-3.2
50	CHOH(4-FC_6H_4)	I			0.1-0.32	0.1-0.32	0.32-1.0
53	CO(3-FC_6H_4)	F	207-209	$C_{27}H_{23}ON_2F_3 \cdot HCl$	10-32	10-32	>32
54	CHOH(3-FC_6H_4)	I	228-230	$C_{27}H_{25}ON_2F_3 \cdot HCl \cdot 0.5H_2O$	0.32-1.0	1.0-3.2	10-32
55	CHOH(2-FC_6H_4)	K	217-219	$C_{27}H_{25}ON_2F_3 \cdot HCl \cdot 0.5H_2O$	0.1-0.32	1.0-3.2	>3.2
56	CO(4-ClC_6H_4)	F	201-203	$C_{27}H_{23}ON_2ClF_2 \cdot HCl$	3.2-10	3.2-10	NT
57	CHOH(4-ClC_6H_4)	I	242-244	$C_{27}H_{25}ON_2ClF_2 \cdot HCl$	1.0-3.2	1.0-3.2	>3.2
58	COC_6H_5	F	208-211	$C_{27}H_{24}ON_2F_2 \cdot HCl \cdot H_2O$	10-32	10-32	NT
59	$CHOHC_6H_5$	I	236-238	$C_{27}H_{26}ON_2F_2 \cdot HCl \cdot 0.5H_2O$	0.32-1.0	0.32-1.0	>3.2
60	CO(4-$CH_3C_6H_4$)	F	125-127	$C_{28}H_{26}ON_2F_2$	>10	>10	NT
61	CHOH(4-$CH_3C_6H_4$)	I	236-237	$C_{28}H_{28}ON_2F_2 \cdot HCl \cdot 0.5H_2O$	0.1-0.32	1.0-3.2	>10
62	CHOH(4-$CH_3OC_6H_4$)	I	163-165	$C_{28}H_{28}O_2N_2F_2 \cdot HCl$[d]	1.0-3.2	1.0-3.2	>3.2
63	CHOH(3-$CF_3C_6H_4$)	I	225-227	$C_{28}H_{25}ON_2F_5 \cdot HCl$	>10	>10	NT
64	CHOH(4-Cl-3-$CF_3C_6H_3$)	I	216-217	$C_{28}H_{24}ON_2ClF_5 \cdot HCl$	>32	>32	>32
65	CHOH(3-pyridyl)	K	175-178	$C_{26}H_{25}ON_3F_2 \cdot 2HCl \cdot 3H_2O$	>3.2	>3.2	NT
66	CO(2-thienyl)	F	241-242	$C_{25}H_{22}ON_2SF_2 \cdot HCl$	10-32	>32	NT
67	CHOH(2-thienyl)	I	142-144	$C_{25}H_{24}ON_2SF_2$	10-32	>32	NT

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table II. [d] N: calcd, 6.41; found, 5.88.

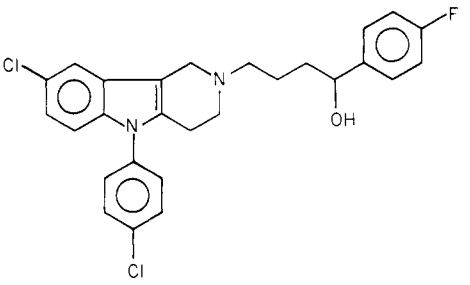
activity of secondary alcohols was found to consistently exceed that of the corresponding ketones within the present series. An important difference between the 5-aryl series presented here and the 5-H or 5-alkyl series II and III is suggested by the reduction in activity seen in going from ketones II or III to the corresponding alcohols IV or V, respectively.



IV, R = H
V, R = CH_3

Having optimized side-chain length and determined that fluorine substitution was preferred at the 4′ and 8 positions, a futher study of the effect upon activity of altering the aryl substitution in the side-chain phenyl ring was undertaken. These results are presented in Table V. Whereas a number of these compounds were active at 1 and 5 h, it will be noted that in all cases, other than **49** and **50**, activity was substantially reduced by 24 h.

Table VI illustrates the effect of varying substitution on the N-5 aryl ring while maintaining optimum substitution at the 2 and 8 positions. Thus, ortho and para substitution in this ring are preferred, with the meta-substituted compounds **68**, **76**, and **77** being less active. Steric factors favor smaller rather than larger substituents, and the effect of substitution here may also be important in modifying rates of metabolism and drug dispositon. Of particular note is the 4′-chloro analogue **71**, which was essentially inactive at 1 and 5 h but was highly active at 24 and 48 h. Surprisingly, the 4′,8-dichloro analogue VI,



VI

which was predicted to display slow onset of action and long duration of action, was inactive in these tests even at doses as high as 32 mg/kg ip and at time periods of up to 72 h.

The preparation of long-chain lipophilic esters of phenothiazine and thioxanthene neuroleptic agents possessing hydroxyl substituents (i.e., fluphenazine enanthate and flupenthixol decanoate) has led to clinically valuable injectable neuroleptic agents with greatly prolonged duration of action.[18,19] Esterification of **50** with a range of carboxylic acids gave the esters presented in Table VII. Of compounds prepared, the pentanoate ester **79** demonstrated the longest duration of action (8–9 days) vs. apo-

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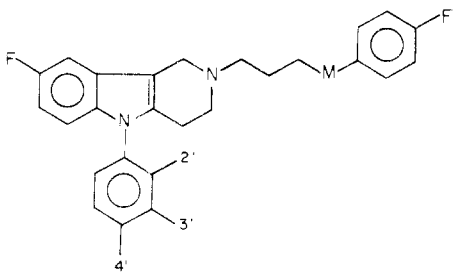
(19) F. J. Ayd, Jr., *Int. Drug Ther. Newsl.*, **7**, 1 (1972).

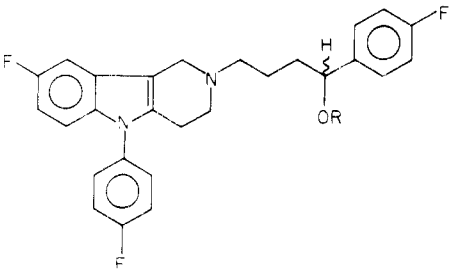
Table VI. Variation in the N-5 Aryl Group Substitution Pattern



no.	2′	3′	4′	M	method[a]	mp, °C	formula[b]	antagonism of amphetamine (rat): ED_{50}, mg/kg ip[c] 1 h	5 h	24 h
49	H	H	F	CO	F			1.0-3.2	1.0-3.2	1.0-3.2
50	H	H	F	CHOH	I			0.1-0.32	0.1-0.32	0.32-1.0
68	H	F	H	CHOH	I	234-235	$C_{27}H_{25}ON_2F_3 \cdot HCl$	3.2-10	>10	NT
69	F	H	H	CHOH	I	229-231	$C_{27}H_{25}ON_2F_3 \cdot HCl$	0.32-1.0	1.0-3.2	>10
70	H	H	H	CHOH	I	223-225	$C_{27}H_{26}ON_2F_2 \cdot HCl \cdot 0.25H_2O$	1.0-3.2	1.0-3.2	3.2-10
71	H	H	Cl	CHOH	I	174-175	$C_{27}H_{25}ON_2ClF_2$	>32	>32	1.0-3.2
72	H	H	CH_3O	CO	F	209-211	$C_{28}H_{26}O_2N_2F_2 \cdot HCl \cdot 0.25H_2O$	10-32	>32	NT
73	H	H	CH_3O	CHOH	I	235-236	$C_{28}H_{28}O_2N_2F_2 \cdot HCl$	0.32-1.0	1.0-3.2	>3.2
74	H	H	CF_3	CO	F	245-247	$C_{28}H_{23}ON_2F_5 \cdot HCl$	>32	NT	NT
75	H	H	CF_3	CHOH	I	248-250	$C_{28}H_{25}ON_2F_5 \cdot HCl$	10-32	10-32	NT
76	H	CF_3	H	CO	F	247-248	$C_{28}H_{23}ON_2F_5 \cdot HCl$	>32	NT	NT
77	H	CF_3	H	CHOH	I	243-245	$C_{28}H_{25}ON_2F_5 \cdot HCl \cdot 0.5H_2O$	>32	>32	NT

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table II.

Table VII. Duration of Action of Lipophilic Esters of Compound 50



no.	OR	method[a]	mp, °C	formula[b]	im dose, mg/kg (vehicle)	apomorphine-induced emesis (dog), days protection[c]
78	$OCOCH_3$	L	oil	$C_{29}H_{27}O_2N_2F_3$	3.2 (sesame oil)	0
79	$OCOC_4H_9$	L	176-178	$C_{32}H_{33}O_2N_2F_3 \cdot HCl \cdot H_2O$	3.2 (sesame oil)	8-9
80	$OCOC_6H_{13}$	L	171-173	$C_{34}H_{37}O_2N_2F_3 \cdot HCl \cdot 0.5H_2O$	2.0 (1 N saline)	1-2
81	$OCOC_9H_{19}$	L	138-139	$C_{37}H_{43}O_2N_2F_3 \cdot HCl$	2.0 (1 N saline)	0
82	$OCOC_{17}H_{35}$	L	oil	$C_{45}H_{59}O_2N_2F_3$[d]	3.2 (sesame oil)	0

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] The ability of each compound to prevent the emesis induced by apomorphine (0.1 mg/kg, iv) in dogs was assessed using the method of Weissman et al.[14] Groups of four dogs were treated intramuscularly with suspensions of compounds in the stated vehicle at the given dose. Apomorphine challenges were administered 5, 24, 48, . . ., h following drug administration. Compounds **78**, **81**, and **82** afforded only partial or no protection at the 5- and 24-h test periods. [d] N: calcd, 3.86; found, 3.35.

morphine-induced emesis in the dog when administered as an im suspension.

In addition to antiamphetamine testing, selected compounds were also examined for their ability to displace bound [^{3}H]spiroperidol from rat corpus striatum homogenates in vitro. This procedure is a common measure of intrinsic affinity for the dopamine receptor, and IC_{50} values from this test have been shown to closely correlate with the clinical potency of a series of antipsychotic drugs.[20] The results presented in Table VIII show that compounds from the present series inhibit spiroperiodol binding with

(20) J. L. Howard, B. T. Large, S. Wedley, and I. A. Pullar, *Life Sci.*, **23**, 599 (1978).

Scheme I

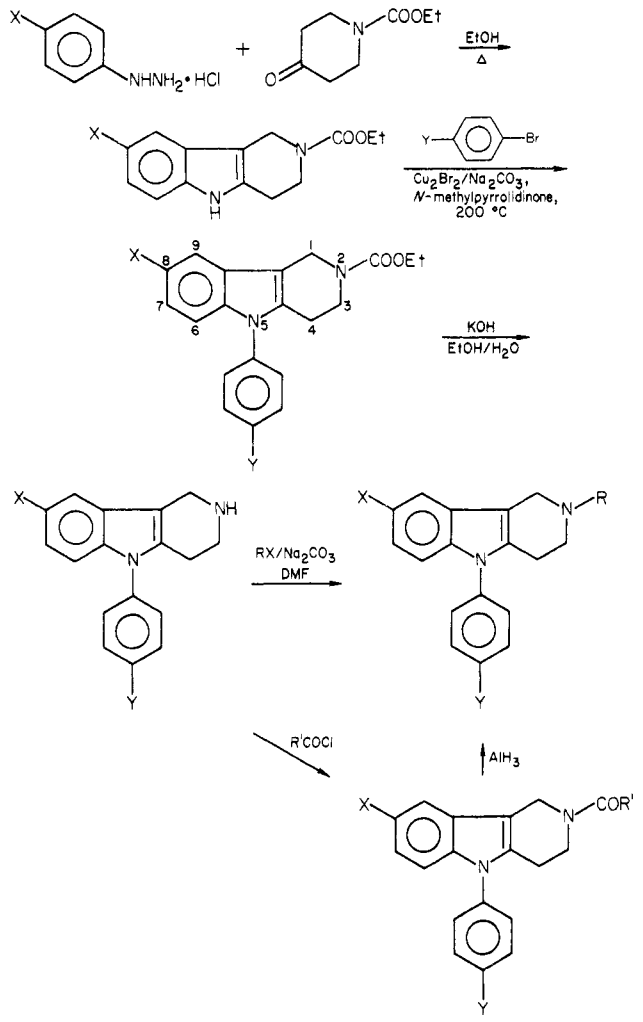


Table VIII. [^{3}H]Spiroperidol Binding

compd	IC_{50}, nM[a]
37[b]	83
49[c]	26
50[c]	14
II[d]	51
III[d]	71
IV[d]	103
V[d]	101
chlorpromazine	51
haloperidol	9

[a] Data were obtained by a modification of the method of Burt, Creese, and Snyder.[29] Details are given under Experimental Section. [b] Structure given in Table III. [c] Structure given in Table IV. [d] Structure given in the text.

approximately the same relative potencies as obtained from the in vivo rat antiamphetamine procedure. Thus, **50** is the most potent compound in vitro, with an IC_{50} in the same range as haloperidol. Similarly, the keto analogue **49** is less potent than **50**. The lower IC_{50} values of **49** and **50** compared with II and IV, respectively, confirm the importance of 5-aryl substitution, as demonstrated previously using in vivo data.

The above findings suggest that the principal determinant of potent activity in the present series is the 5-aryl-γ-carboline nucleus and not the 2 substituent. Although butyrophenone and reduced butyrophenone moieties at position 2 contribute to potency and duration of action, they are not essential for neuroleptic activity. This is most clearly shown by the good antiamphetamine activity of the N-2 alkyl derivatives, particularly the *N*-methyl analogue (**37**). Furthermore, whereas reduction of the butyrophenone side chain in butyrophenone neuroleptics typically lends to diminished activity,[21] analogues with reduced butyrophenone side chains are markedly more active in the 5-aryltetrahydro-γ-carboline series.

The potent in vitro and in vivo activity of the 5-aryl-1,2,3,4-tetrahydro-γ-carbolines, which are conformationally restrained in a semirigid nucleus, allows certain conclusions to be made regarding conformational requirements for dopamine receptor blockade. As discussed in our earlier communication,[9] the relative spatial relationship of the indole-fused aromatic ring and the basic nitrogen in the tetrahydro-γ-carbolines appears to approximate optimum values for interaction with the dopamine receptor. In compound **50**, the distance between the center of the indole-fused aromatic ring and the basic nitrogen has been determined by X-ray crystallography to be 5.16 Å,[22] which is in good agreement with the values of 5.10 and 5.12 Å published for (+)-dexclamol[23] and apomorphine,[24] respectively. Similarly, the distance of the basic nitrogen atom out of the plane defined by the indole nucleus is ±0.6 Å, again in good agreement with the figure of −0.90 Å published for (+)-dexclamol.[23]

The relative importance of the aromatic ring to basic nitrogen distance and the nitrogen out-of-plane distance is not clear at this time. It can be noted, however, that isobutaclamol, which is as potent as butaclamol as a neuroleptic, has recently been reported[25] to have values of 6.44 and ±0.9 Å, respectively. These results and the data presented herein suggest that the out-of-plane distance may be the more critical parameter, but more data from other active neuroleptics will be required before firm conclusions can be made in this regard.

Compound **50** (CP-36,584; USAN name, flutroline), the most potent and long-acting agent of those shown in Table IV, displays a full range of neuroleptic effects in animals, including selective blockade of conditioned avoidance behavior, blockade of apomorphine- and amphetamine-elicited symptoms in several species, inhibition of [^{3}H]-spiroperidol binding in brain, and evidence of potent enhancement of dopamine turnover.[26] This compound is currently undergoing clinical evaluation as an antipsychotic agent.

Experimental Section

Melting points (uncorrected) were taken with a Thomas-Hoover capillary apparatus. NMR spectra were recorded on Varian A-60, T-60, and XL-100 spectrometers with Me_4Si as an internal standard. IR spectra were determined with a Perkin-Elmer Model 21 spectrometer. UV spectra were recorded on a Carey Model 14 spectrophotometer. Mass spectra were obtained with a Perkin-Elmer RMU-6E mass spectrometer. Microanalyses were performed by the Pfizer Analytical Department. Many of the compounds described in this paper, particularly HCl salts, retained a partial mole of H_2O despite drying in vacuo. This was confirmed by Karl-Fischer determination in several cases.

(21) (a) A. G. Rauws, M. Olling, J. Freudenthal, and M. ten Ham, *Toxicol. Appl. Pharmacol.*, **35**, 333 (1976); (b) C. H. Grogan, C. F. Geshickter, M. E. Freed, and L. M. Rice, *J. Med. Chem.*, **8**, 62 (1965).
(22) J. Bordner, J. J. Plattner, and W. M. Welch, *Cryst. Struct. Commun.*, submitted.
(23) P. H. Bird, F. T. Bruderlein, and L. G. Humber, *Can. J. Chem.*, **54**, 2715 (1976).
(24) J. Giesecke, *Acta Crystallogr., Sect. B*, **29**, 1985 (1973).
(25) A. H. Philipp, L. G. Humber, and K. Voith, *J. Med. Chem.*, **22**, 768 (1979).
(26) A. Weissman and B. K. Koe, to be published.

Method A. Preparation of 2-Carbethoxy-1,2,3,4-tetrahydro-γ-carbolines. 4-Fluorophenylhydrazine hydrochloride (48.6 g, 0.8 mol) and 1-carbethoxy-4-piperidinone (51.3 g, 0.3 mol) were heated at relux in ethanol (225 mL) for 1.75 h and then allowed to stand at room temperature overnight. The solid product was filtered and washed with 50% aqueous ethanol. After drying at 50 °C (25 mm), there was obtained 61.1 g (69%) of **3**, which was used as such in the next step. Recrystallization from aqueous ethanol provided an analytical sample, mp 169–170 °C.

Method B. Arylation of 2-Carbethoxy-1,2,3,4-tetrahydro-γ-carbolines. 2-Carbethoxy-8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (14). A mixture of 2-carbethoxy-8-fluoro-1,2,3,4-tetrahydro-γ-carboline (60.3 g, 0.23 mol), 4-bromofluorobenzene (136.8 g, 0.782 mol), cuprous bromide (72.3 g, 0.253 mol), and sodium carbonate (26.6 g, 0.253 mol) in 450 mL of dry *N*-methyl-2-pyrrolidinone was heated with vigorous stirring at 200 °C for 8 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 600 mL of water and 125 mL of ethylenediamine. Benzene (250 mL) was then added, and the resulting mixture was filtered through diatomaceous earth. The filtrate was then extracted several times with benzene, and the combined extracts were washed with water, dried, and evaporated to yield an amber oil. Chromatography of the oil on silica gel (eluting with benzene), followed by evaporation of the solvent, gave 63.8 g (78%) of crystalline **14**, mp 121–123 °C.

Method C. Acylation of 5-Aryl-1,2,3,4-tetrahydro-γ-carbolines. 2-Benzoyl-8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (19; Table I). To a cooled solution of 8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (**26**; 14 g, 4.9 mol) in 10 mL of methylene chloride and 2 mL of triethylamine was added a solution of benzoyl chloride (7.5 g, 5.42 mmol) in 5 mL of methylene chloride. The reaction mixture was allowed to warm to room temperature over a period of 1 h and then poured into aqueous sodium bicarbonate. Extraction with methylene chloride, followed by washing, drying, and solvent removal, gave 1.55 g (97%) of **19**, mp 141–144 °C.

Method D. Hydrolysis of 2-Carbethoxy-5-aryl-1,2,3,4-tetrahydro-γ-carbolines. 5-(4-Fluorophenyl)-8-fluoro-1,2,3,4-tetrahydro-γ-carboline (26; Table I). A suspension of 2-carbethoxy-8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (**14**; 3.56 g, 0.01 mol) in 25 mL of ethanol was added to a solution of potassium hydroxide (11.2 g, 0.20 mol) in 35 mL of ethanol and 5 mL of water. The resulting solution was heated at reflux under nitrogen for 23 h. The dark solution was concentrated in vacuo, diluted with water, and extracted with ether. Drying of the ether extracts, followed by evaporation, gave 2.6 g of **26**, mp 125–127 °C. An analytical sample was prepared by recrystallization from cyclohexane, mp 127–128 °C.

Method E. Reduction of 2-Acyl-5-aryl-1,2,3,4-tetrahydro-γ-carbolines. 8-Fluoro-5-(4-fluorophenyl)-2-methyl-1,2,3,4-tetrahydro-γ-carboline (37; Table III). Aluminum hydride was generated in situ by the addition of aluminum chloride (5.2 g, 0.039 mol) to a cold solution of lithium aluminum hydride (4.48 g, 0.118 mol) in 150 mL of ether. The resulting suspension was stirred at 0–5 °C for 5 min and then a solution of 2-carbethoxy-8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (**14**; 17.5 g, 0.049 mol) in 90 mL of ether and 100 mL of tetrahydrofuran was added dropwise. After the solution was stirred at 5–15 °C for 30 min, the excess aluminum hydride was destroyed with water and the precipitated aluminum salts were removed by filtration. Evaporation of the filtrate left 9.6 g of solid, mp 124–127 °C. Hydrogen chloride gas was passed into an ethereal solution of this solid, affording 9.9 g (60%) of salt, mp 295–296 °C.

Method F. Alkylation of 5-Aryl-1,2,3,4-tetrahydro-γ-carbolines. 8-Fluoro-5-(4-fluorophenyl)-2-phenethyl-1,2,3,4-tetrahydro-γ-carboline (42; Table IV). A stirred mixture of 8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (**26**; 2.84 g, 0.01 mol), phenethyl bromide (259 g, 0.014 mol), anhydrous sodium carbonate (3.15 g, 0.03 mol), dimethylformamide (50 mL), and a trace of potassium iodide was heated at 65 °C under nitrogen for 4 h, cooled, poured into water, and extracted with ether. The extract was dried (magnesium sulfate) and concentrated in vacuo. The resulting oil crystallized following trituration with hexane. Passage of hydrogen chloride gas through an ethereal solution of the solid afforded the hydrochloride salt, which, after recrystallization from ethanol–ether, gave 2.3 g (54%) of **37**, mp 275–276 °C.

Method G. Dehydration of Secondary Alcohols. 8-Fluoro-5-(4-fluorophenyl)-2-[4-(4-fluorophenyl)but-3-enyl]-1,2,3,4-tetrahydro-γ-carboline (43; Table IV). A solution of 2.0 g (41 mmol) of compound **50** in 50 mL of 6 N HCl and 20 mL of EtOH was heated at reflux for 4 h and was then stirred at room temperature overnight. The white solid which separated was isolated by filtration, air-dried, and was then recrystallized twice from EtOH to give 0.71 g (37%) of compound **43**, mp 258–259 °C.

Method H. Reduction of Alkenyl Side Chains. 8-Fluoro-5-(4-fluorophenyl)-2-[4,4-bis(4-fluorophenyl)butyl]-1,2,3,4-tetrahydro-γ-carboline (46; Table IV). A solution of 1.50 g (2.85 mmol) of compound **45** in 50 mL of glacial acetic acid was treated with 80 mg of PtO_2, and the mixture was reduced at 50 psi of hydrogen for 4.5 h. The catalyst was filtered and the solvent was evaporated. The residues were partitioned between CH_2Cl_2 and 1 N NaOH, and the organic phase was then dried and evaporated to an amorphous solid, which was converted to the HCl salt in ethyl acetate to give 800 mg of compound **46**, mp 224–226 °C.

Method I. Preparation of Secondary Alcohols. 8-Fluoro-5-(4-fluorophenyl)-2-[4-(4-fluorophenyl)-4-hydroxybutyl]-1,2,3,4-tetrahydro-γ-carboline Hydrochloride (50; Table IV). A solution of 9.41 g (21 mmol) of the ketone **49** in 100 mL of 1:1 EtOH–THF was added dropwise to a stirred suspension of $NaBH_4$ (3.17 g, 84 mmol) in 50 mL of EtOH. After the addition was complete, the reaction mixture was stirred for an additional 30 min and then poured into 150 mL of cold water. Volatile solvents were removed on a rotary evaporator, leaving a solid precipitate which was collected and then dissolved in CH_2Cl_2 and dried. Evaporation of the solvent left an oil, which was dissolved in ether and treated with HCl (gas) to yield the hydrochloride salt, which was recrystallized from methanol: yield 9.4 g (92%); mp 246–247 °C after drying overnight at 65 °C (25 mm).

Method J. Acylation of 5-Aryl-1,2,3,4-tetrahydro-γ-carbolines. 8-Fluoro-5-(4-fluorophenyl)-2-[4-oxo-4-(4-fluorophenyl)butyryl]-1,2,3,4-tetrahydro-γ-carboline. A solution of 690 mg (3.52 mmol) of 4-fluorobenzoylpropionic acid in 10 mL of CH_2Cl_2 was cooled to 0 °C. To this was added a solution of 725 mg (3.52 mmol) of dicyclohexylcarbodiimide in 5 mL of ice-cold CH_2Cl_2. After 20 min, a solution of 8-fluoro-5-(4-fluorophenyl)-1,2,3,4-tetrahydro-γ-carboline (**26**; 1.0 g, 2.54 mmol) in 7 mL of cold CH_2Cl_2 was added to the milky suspension over a period of about 20 min. The reaction mixture was allowed to warm to room temperature and then stirred for 2–4 h. Then the mixture was again cooled to 0 °C, and dicyclohexylurea was separated by filtration. The filtrate was evaporated in vacuo and the residues were washed through a 20-g column of silica gel with 1:1 benzene–ethyl acetate. The product crystallized from ethanol to yield the desired product in 92% yield, mp 153.0–154.5 °C.

Method K. Reduction of Keto Amides. A filtered solution of lithium aluminum hydride in THF was added dropwise to a solution of 750 mg (1.62 mmol) of the above keto amide in 25 mL of dry THF at reflux. A rapid foaming occurred with each addition. When foaming no longer occurred with the addition of the reagent, the amount of $LiAlH_4$ was judged to be sufficient. The reaction mixture was then heated at reflux for an additional 5 min and then cooled and poured onto 150 mL of ice. Aluminum salts were filtered off and washed with ether. The filtrate was then separated, and the aqueous phase was washed once with ether. The combined ether extracts were dried and treated with an ether solution of HCl gas to give colorless crystals of **50**·HCl, identical in every respect with the compound prepared by method I above.

Method L. Esters of 50. Preparation of 81 (Table VII). A solution of 973 mg (2.0 mmol) of **50**·HCl in 25 mL of THF and 15 mL of CH_2Cl_2 was treated at room temperature with 444 mg (4.4 mmol) of triethylamine and 400 mg (2.1 mmol) of decanoyl chloride. The reaction mixture was stirred for 2 h and then poured into 150 mL of H_2O. This mixture was extracted several times with ether, and the combined ether extracts were then back washed with 2% HCl and 2% NaOH. The residues remaining

after drying and evaporation of the solvent were chromatographed on 25 g of silica gel using 1:1 benzene–ethyl acetate as eluent. the desired product crystallized slowly from ether–pentane to give 0.648 g (51%) of colorless crystals, mp 138–139 °C.

Biological Methods. Antagonism of *d*-Amphetamine-Induced Symptoms in Rats. Neuroleptic effects in vivo were estimated by the blockade of amphetamine stereotypy. Rats were placed individually in covered plastic compartments; after a brief period of acclimation in the cages, the rats in groups of five were treated intraperitoneally with compounds at doses separated by 0.5 log unit (i.e., ..., 1, 3.2, 10, 32, ... mg/kg). They were subsequently treated 1, 5, and 24 h later with *d*-amphetamine sulfate, 5 mg/kg ip. One hour after each amphetamine challenge, each rat was assessed for its most characteristic behavior on a six-point scale.[27] These ratings represent increasing degrees of drug effect,[28] and the time of rating chosen coincides with the peak effect of amphetamine.[14] Scores were dichotomized (cf. ref 27), and approximate ED_{50} values were determined, based on the quantal data. Doses are expressed in terms of the hydrochloride salts.

[^{3}H]Spiroperidol Binding to Dopamine Receptor. The method was adapted from that of Burt, Creese, and Snyder.[29] Rats (Sprague–Dawley CD males, 250–300 g, Charles River Laboratories, Wilmington, MA) were decapitated, and brains were immediately dissected to recover the corpus striatum. The latter was homogenized in 40 volumes of ice-cold 50 mM Tris-HCl [tris(hydroxymethyl)aminomethane hydrochloride] buffer, pH 7.7, with a Brinkmann Polytron PT-10. The homogenate was centrifuged twice at 50000*g* for 10 min at 0–4 °C with rehomogenization of the intermediate pellet in fresh Tris buffer (same volume) in the Polytron., The final pellet was gently resuspended in 90 volumes of cold 50 mM Tris-HCl buffer, pH 7.6, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline. The tissue suspension was placed in a 37 °C water bath for 5 min and kept ice cold until use. The incubation mixture consisted of 0.02 mL of inhibitor solution or vehicle, 1.0 mL of tissue preparation, and 0.10 mL of [^{3}H]spiroperidol solution (New England Nuclear, 23.6 Ci/mmol), prepared so as to obtain a 0.5 nM final concentration. Tubes were incubated in sequence for 10 min at 37 °C in groups of three, after which 0.9 mL from each incubation tube was filtered through Whatman GF/B filters under vacuum. After washing twice with 5 mL of cold Tris-HCl, pH 7.7, buffer, each filter was placed in a scintillation vial with 10 mL of Aquasol-2 (New England Nuclear), and each vial was vortexed. Samples were kept at room temperature overnight before determination of radioactivity in a liquid scintillation counter. Binding was calculated as fmol of [^{3}H]spiroperidol bound/mg of protein. Controls (vehicle or 10^{-7} M *l*-butaclamol), blank (10^{-7} M *d*-butaclamol), and inhibitor solutions (four concentrations) were run in triplicate. The concentration that reduced binding by 50% (IC_{50}) was estimated on semilog paper. The IC_{50} values in Table I represent means of two to three runs. Insoluble drugs were dissolved in ethanol (1–2% ethanol in final incubation mixture).

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8-Chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepines with Substituents at C-4

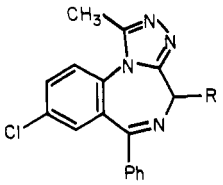
Jackson B. Hester, Jr.,* Allan D. Rudzik, and Philip F. VonVoigtlander

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001. *Received December 7, 1979*

A series of 8-chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepines with substituents at C-4 was prepared and evaluated for antianxiety potential. It was found that substitution at this position generally decreased the activity in this series.

Our interest in the antianxiety activity of the 1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepines [viz., alprazolam (1)][1,2] prompted us to study the structure–activity relationships of members of this series with substituents at C-4. Of particular interest in this regard were the 4-hydroxy derivatives (viz., 8) which, based on the experience with diazepam,[3] were potential metabolites. In this report we present our methods for the synthesis of these compounds and their pharmacological activity.

1



2, R = $COOC_2H_5$
3, R = CH_3
4, R = NH_2
5, R = CH_2COOH
6, R = CH_2CH_2COOH
7, R = $OC(=O)CH_3$
8, R = OH

Our discovery that the carboxylic acid derived from ester 2 was easily decarboxylated made it possible to activate the 4 position of 1 for electrophilic reactions via the ester. Subsequent hydrolysis and decarboxylation of this activating function would then give the desired monosubstituted products. The ester (2) was prepared in 56% yield by the reaction of 1 with diethyl carbonate and sodium hydride. Alkylation was readily accomplished by the reaction of 2 with sodium hydride and an appropriate alkyl halide. Thus, with methyl iodide, 9 was obtained in 72% yield; subsequent sodium hydroxide hydrolysis, followed by decarboxylation of the acid, gave 3 in 63% yield. Compounds 5 and 6 were prepared in a similar manner. The reaction of 2 with *O*-(2,4-dinitrophenyl)hydroxylamine and sodium hydride gave 10 in 77% yield. Sodium hy-

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