A Pharmacological Investigation of Potential Antidepressants of the Amitriptyline-Type

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The hope of finding compounds with clinical antidepressant properties of the imipramine type, led to the synthesis and pharmacological investigation of a new series of compounds, dibenzo[a,d]-cycloheptadiene derivatives.[1-3]

The intensive pharmacological study of one of these compounds, amitriptyline,[4,5] and the subsequent clinical verification of its antidepressant properties[6-8] has provided some information about the types of pharmacological effects in animals which are predictive of antidepressant activity in humans. It should be made clear, however, that there is to date no single test which is indicative of antidepressant activity; rather, it has been the profile of pharmacological activity and the similarity of chemical structure which has led to the clinical trial of new compounds in this area.

The compounds reported here were synthesized in this laboratory for pharmacological investigation as psychotropic agents.[1,9] Amitriptyline (compound 3) and desmethyl-amitriptyline[10] (compound 5) are included in the series. The compounds were studied by several methods for both central and peripheral activities.

Methods.—The compounds were tested for acute toxicity (LD_{50}), effect on rectal body temperature, effect on maximal electroshock seizures (MES), potentiation of a subhypnotic dose of alcohol, effect on a trained runway response, ataxic effect, and effect on a conditioned avoidance response. A full description of these experimental procedures has been reported previously.[5] The mydriatic effect (change in pupil diameter caused by 1/4 LD_{50} in mice) was also measured. The results were expressed as the mean difference between the pre- and post-treatment score (arbitrary units measured by means of a scale mounted within a stereomicroscope). A score of 40 represented maximal dilation of the pupil. All compounds were injected i.p. When used, the LD_{50} and ED_{50} were calculated by the method of Litchfield and Wilcoxon.[11]

The antispasmodic activity of the compounds *in vitro* (concentration which inhibited the contraction by 50%) was studied on isolated strips of guinea pig ileum using one of two spasmogens (0.1 μg./ml. of acetylcholine or 0.1 μg./ml. of histamine dihydrochloride).

The results are presented in Table I. Unless otherwise indicated, the compounds were studied as the HCl salt, however, the results are expressed as the free base in all cases.

Results and Discussion

The compounds studied were dibenzo [a,d]-cycloheptadiene and -triene derivatives having side chains which have been applied in the phenothiazine, thioxanthene and iminodibenzyl series. In general, it can be stated that the compounds with those side chains which had proven active in the other series were also effective in our series. Representative compounds from each of these series, chlorpromazine, chlorprothixene, imipramine, and amitriptyline have been studied and compared[1,5] and it should be emphasized that, although the two latter compounds are called "antidepressants" clinically, they do not possess classical stimulating properties in animals. They possess, rather, depressant properties, *e.g.*, they lower body temperature, potentiate narcosis, depress exploratory motility and impair learned responses. It has been pointed out previously that it might be their relative effectiveness on different tests which could best characterize their psychotropic action. It was, therefore, the profile of pharmacological activity rather than the effectiveness of a compound on any particular test which was studied in this series. It can be seen from Table I that most of the compounds had some activity in every test, but seldom did a particular structural modification lead to uniform alteration in activity in all tests. This fact limits the possibility of drawing precise structure-activity relationships. There are, however, a few generalizations which can be made.

When the three main chemical classes (tertiary carbinols, olefins, and their corresponding saturated forms, represented by compounds 1, 3, and 2, respectively) were compared, the tertiary carbinols, *e.g.*, compounds 1, 19, and 24, were the least potent both centrally and peripherally, whereas the corresponding olefins, *e.g.*, compounds 3, 21, and 26, were generally the most potent.

The introduction of a double-bond in the 10,11 position of the ring (R = CH=CH, compounds 42–47) caused an increase in central activity (as indicated by alcohol potentiation and runway effect) and a decrease in peripheral activity. Compounds in which the 3-position was substituted by chlorine had pharmacological properties more similar to the tranquilizer, chlorpromazine, than to the antidepressants. Two compounds, 41 and 45, are most striking in this respect. They caused marked hypothermia, and potentiated alcohol narcosis in very small doses.[12] Furthermore, the effective dose range of these compounds on a conditioned avoidance response was closer to that of chlorpromazine than to that of either imipramine or amitriptyline.[5] Finally, it was found that compounds with a chlorine in the 3-position depressed spontaneous motility in doses of 4–8 mg./kg., i.p., whereas, similar depression was seldom obtained with doses as high as 1/4 LD_{50} in the unsubstituted compounds. A characterization of these compounds as tranquilizer-like is in agreement with the statement of Protiva, *et al.*[2]

In conclusion, it can be stated that many of the

(1) S. O. Winthrop, M. A. Davis, G. S. Myers, J. G. Gavin, R. Thomas, and R. Barber, *J. Org. Chem.*, **27**, 230 (1962).

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(3) F. J. Villani, C. A. Ellis, C. Teichman, and C. Bigos, *ibid., Chem.*, **5**, 373 (1962).

(4) V. G. Vernier, *Dis. Nerv. Sys.*, **22**, 87 (1961).

(5) F. Herr, J. Stewart, and M.-P. Charest, *Arch. Intern. Pharmacodyn.*, **134**, 328 (1961).

(6) W. Dorfman, *Psychosomatics*, **1**, 153 (1960).

(7) F. J. Ayd Jr., *ibid.*, **1**, 320 (1960).

(8) H. Freed, *Am. J. Psychiat.*, **117**, 455 (1960).

(9) Compound 4 was prepared by Dr. M. A. Davis through the interaction of 5-(3-bromopropylidene) dibenzo [a,d]-cycloheptadiene[1] with O,N-dimethylhydroxylamine [R. T. Major and E. E. Fleck, *J. Am. Chem. Soc.*, **50**, 1479 (1928)] in boiling ethanol. The hydrochloride had m.p. 115–116° (from 2-propanol-ether). *Anal.* Calcd. for $C_{20}H_{24}ClNO$: C, 72.81; H, 7.33; Cl, 10.74; N, 4.25. Found: C, 72.59; H, 7.26; Cl, 10.41; N, 4.10.

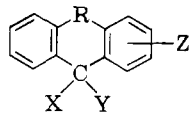
(10) S. Garattini, A. Giachetti, A. Jori, L. Pieri, and L. Valzelli, *J. Pharm. Pharmacol.*, **14**, 509 (1962).

(11) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

(12) In our experiments chlorpromazine lowered body temperature 8.6° at 1/4 LD_{50}, i.p., in mice, and the ED_{50} for alcohol potentiation was 0.5 ± 0.08 mg./kg., i.p.

compounds possessed profiles of pharmacological activity similar to that of amitriptyline and, therefore, they could be expected to possess antidepressant properties in depressed patients. The final characterization of the psychotropic properties of these compounds must await clinical studies.

TABLE I



Compound no.	X	Y	Z	LD50 approx. mg./kg. (mice)	Temperature decrease °C mice (*rats) 1/4 LD50	Mydriasis 1/4 LD50 (mice)	MES ED50 mg./kg. (mice)	Alcohol potentiation ED50 mg./kg. (mice)	Runway effect approx. ED50 mg./kg. (rats)	Ataxia approx. ED50 mg./kg. (rats)	Guinea Pig Ileum Anti-Ach relative potency; atropine = 100	Guinea Pig Ileum Antihistamine relative potency; promethazine = 100
	R = CH_2—CH_2											
1	OH	$(CH_2)_3N(CH_3)_2$	H	120–130	3	7	29 ± 0.7	33 ± 2	30	55	0.27	4.5
2	H	$(CH_2)_3N(CH_3)_2$	H	112 ± 3	5	31	15.5 ± 1	10 ± 0.2	11	40	35	114
3	=CH	$CH_2CH_2N(CH_3)_2$	H	83 ± 2	3.5	19	10 ± 0.8	7.9 ± 0.8	8	53	9.5	80
4	=CH	$CH_2CH_2N(CH_3)OCH_3$	H	400–450	..	3.5	>100	83 ± 7	..	..	0.03	0.14
5	=CH	$CH_2CH_2NHCH_3$	H	70–80	..	10	19 ± 3	7.5 ± 1	8	24	1.4	40
6	H	$(CH_2)_3N(C_2H_5)_2$	H	60–70	2.5	..	17 ± 1	13 ± 2	9	45	5	27
7	=CH	$CH_2CH_2N(C_2H_5)_2$	H	55–65	4.1	16.8	16.5 ± 1	14 ± 1.4	14	25	22	31
8	OH	$(CH_2)_3NC_5H_{10}$[a]	H	180–210	0.8*	2	25 ± 4	22 ± 5	..	>90	0.3	2
9	H	$(CH_2)_3NC_5H_{10}$[a]	H	80–90	0.6	14	22 ± 1	10.5 ± 1	16	45	1.3	33
10	=CH	$CH_2CH_2NC_5H_{10}$[a]	H	80–90	4.1	10.8	16.5 ± 0.3	11 ± 0.5	15	>40	1.6	30
11	=CH	$CH_2CH_2NC_6H_{12}$[b]	H	90–110	1.6*	17.4	13.5 ± 1.2	12 ± 1.4	12	70	4.5	15
12	=CH	$CH_2CH_2NC_5H_8$[c]	H	90–110	2*	24	13.5 ± 0.9	15 ± 1.8	8	45	11	22
13	=CH	$CH_2CH_2N_2C_5H_{11}$[d]	H[o]	115–125	2.1*	3.8	20.5 ± 1	17 ± 1.1	8	50	0.4	40
14	=CH	$CH_2CH_2N_2C_6H_{13}O$[e]	H[o]	100–110	5.6	5.4	33 ± 1.4	12.5 ± 1.3	8	50	0.2	6.9
15	=CH	$CH_2CH_2N_2C_8H_{15}O_2$[f]	H[o]	130–150	2.3*	0	30 ± 1.2	8.5 ± 1.7	6	45	1.2	6.8
16	OH	$(CH_2)_3NC_4H_8O$[g]	H	270–320	1.1*	2	106 ± 9	44 ± 2	..	..	0.09	2.2
17	=CH	$CH_2CH_2NC_4H_8O$[g]	H	180–220	2.1*	10.8	36 ± 1.8	23 ± 1.8	18	62	2.8	36
18	=CH	$CH_2CH_2NC_{14}H_{19}O_2$[h]	H	210–230	..	0	>60	1.1 ± 0.1	18	90	..	..
19	OH	$CH_2CH(CH_3)CH_2N(CH_3)_2$	H	220–270	1.4*	5	32 ± 0.9	>53	..	>110	0.13	2
20	H	$CH_2CH(CH_3)CH_2N(CH_3)_2$	H	110–135	3.2	21	22 ± 1	22 ± 2	10	35	10	57
21	=CH	$CH(CH_3)CH_2N(CH_3)_2$	H	80–90	2.1	9.4	20 ± 1	>22	15	35	2.3	29
22	H	$CH_2C(CH_3)_2CH_2N(CH_3)_2$	H	1 5–145	..	14.4	27.5 ± 1.4	24 ± 5	20	60	6.5	16
23	=CH	$C(CH_3)_2CH_2N(CH_3)_2$	H	115–125	1*	8.1	27 ± 1.4	20 ± 1.4	10	65	13	13
24	OH	$CH_2CH_2N(CH_3)_2$	H	160–175	2.1*	6	23 ± 1.6	21 ± 1.3	22	60	<0.2	9.8
25	H	$CH_2CH_2N(CH_3)_2$	H	80–90	2.4*	13	13 ± 0.6	12 ± 1.5	13	30	3	220
26	=CH	$CH_2N(CH_3)_2$	H	75–90	2.3*	19	9.8 ± 0.9	14 ± 2	13	30	12	98
27	OH	$CH_2CH_2N_2C_5H_{11}$[d]	H[p]	60–70	0	0	>15	18 ± 3	10	47	0.4	6
28	H	$CH_2CH_2N_2C_5H_{11}$[d]	H	115–125	1.9*	2.1	34 ± 8	26 ± 3	23	66	1.5	20
29	=CH	$CH_2N_2C_5H_{11}$[d]	H[p]	75–80	1*	15	22 ± 6	27 ± 5	13	55	17	15
30	=CH	$CH_2C_6H_{12}N$[i]	H[q]	95–115	1.5	17	17 ± 2.2	10.5 ± 1	12	40	13	40
31	OH	$CH_2C_5H_{10}N$[j]	H	105–125	0*	4.4	>35	13 ± 1.4	24	>90	0.2	9
32	H	$CH_2C_5H_{10}N$[j]	H[r]	70–75	1*	35	21 ± 1	5 ± 1	7	40	..	..
33	=CH	$C_5H_{10}N$[j]	H[r]	55–60	0*	25.8	17 ± 2	0.9 ± 0.2	8	26	40	67
34	OH	$CH_2CH_2C_6H_{12}N$[k]	H	90–100	0*	6	34 ± 3	21.6 ± 2	22	70	8	4.5
35	H	$CH_2CH_2C_6H_{12}N$[k]	H	80–90	0*	33	27 ± 2	13 ± 4	6	55	200	111
36	=CH	$CH_2C_6H_{12}N$[k]	H[q]	60–75	2.3*	24	16.7 ± 2	6 ± 0.6	3	30	57	50
37	H	$CH_2C_5H_{10}N$[l]	H	45–55	..	0	>15	13.7 ± 2	10	25	0.9	6
38	=CH	$CH_2CH_2N(CH_3)_2$	2-Cl	90–115	2.3*	0.8	37 ± 1	15 ± 3	18	90	..	..
39	H	$CH_2CH_2CH_2N(CH_3)_2$	3-Cl	70–90	4	8	>15	>15	13	45	0.6	34
40	=CH	$CH_2CH_2N(CH_3)_2(\alpha)$[m]	3-Cl	90–110	4.9	15.8	22 ± 1.2	17 ± 2	10	>40	9.5	16
41	=CH	$CH_2CH_2N(CH_3)_2(\beta)$[n]	3-Cl	80–90	7.4	..	20 ± 2.4	1.5	10	30	..	..
	R = CH=CH											
42	=CH	$CH_2CH_2N(CH_3)_2$	H	90–100	6.9	..	19.2 ± 1.4	5.7 ± 0.8	9	45	14.8	66
43	OH	$(CH_2)_3N(CH_3)_2$	3-Cl[s]	85–95	1.7*	2.6	22 ± 1.7	17 ± 2.6	18	50	1.6	3
44	=CH	$CH_2CH_2N(CH_3)_2(\alpha)$[m]	3-Cl	90–110	7.1	24.6	25 ± 0.5	10 ± 1	8	50	8	15
45	=CH	$CH_2CH_2N(CH_3)_2(\beta)$[n]	3-Cl	90–110	11	..	20 ± 0.8	1.7 ± 0.5	5	22	3	18
46	=CH	$CH(CH_3)CH_2N(CH_3)_2$	3-Cl	117 ± 3.6	2.5*	2.8	29 ± 1.5	11 ± 0.9	7	45	19	3
47	=CH	$CH_2CH_2N(CH_3)_2$	2,3-$(OCH_3)_2$	65–75	0*	1.8	>20	15.8 ± 1.3	20	>55	0.7	0.7

[a] NC_5H_{10} = piperidino. [b] NC_6H_{12} = 2 methylpiperidino. [c] NC_5H_8 = 1,2,5,6 tetrahydropyridino. [d] $N_2C_5H_{11}$ = 4-methylpiperazino. [e] $N_2C_6H_{13}O$ = 4-(2-hydroxyethyl)piperazino. [f] $N_2C_8H_{15}O_2$ = 4-(2-acetoxyethyl)piperazino. [g] NC_4H_8O = morpholino. [h] $NC_{14}H_{18}O_2$ = 4-carbethoxy-4-phenylpiperidino. [i] $C_6H_{12}N$ = 1-methyl-3-piperidyl. [j] $C_5H_{10}N$ = 1-methyl-3-pyrrolidyl. [k] $C_6H_{12}N$ = 1-methyl-2-piperidyl. [l] $C_5H_{10}N$ = 2-piperidyl. [m] higher melting point geometric isomer. [n] lower melting point geometric isomer. [o] 2HCl. [p] base. [q] H_2SO_4. [r] oxalate. [s] H_3PO_4.