Examination of a Series of 8-[3-[Bis(4-fluorophenyl)amino]propyl]-1-aryl-1,3,8-triazaspiro[4.5]decan-4-ones as Potential Antipsychotic Agents

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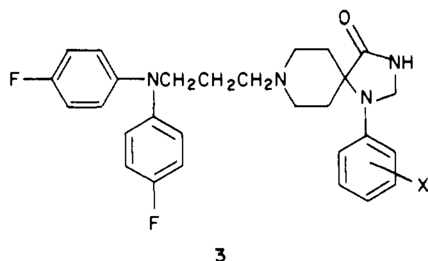
8-[3-[Bis(4-fluorophenyl)amino]propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**3**) and related compounds have been shown to have antipsychotic profiles in biochemical and behavioral pharmacological test models. The dose of **3** necessary to produce catalepsy in rats is much greater than that required for activity in behavioral tests predictive of antipsychotic efficacy, for example the suppression of high base line medial forebrain bundle self-stimulation in rats. This suggests that **3** would have a reduced propensity for neurological side effects. The effects of substitution on the 1-phenyl moiety and on the N-3 nitrogen atom of the triazaspirodecanone portion of **3** were examined. Results from this study suggest that behavioral activity is sensitive to substituents on the 1-phenyl moiety while substituents on the N-3 nitrogen are more generally tolerated. In both rats and squirrel monkeys compound **3** was found to have a similar separation between doses inhibiting Sidman avoidance activity and those causing catalepsy. However, in an extrapyramidal side effect (EPS) test model using haloperidol-sensitized cebus monkeys, **3** elicited signs of EPS at doses approximating those previously determined to be efficacious.

In our previous paper we described the synthesis and structure–activity studies of a series of 1-[3-(diarylamino)propyl]piperidines and related analogues.[1] It was demonstrated that compounds of this series both are quite active in behavioral tests predictive of antipsychotic efficacy and bind to the dopamine receptor in vitro. However, unlike the related (diarylbutyl)piperidine antipsychotics, some of these 1-[3-(diarylamino)propyl]piperidines show a separation between active doses in tests predictive of therapeutic efficacy and extrapyramidal side effects (EPS) as measured in rats by the suppression of the high base line medial forebrain bundle self-stimulation test and the catalepsy test, respectively. These data suggest that these compounds, like the atypical antipsychotic agents such as clozapine, might have a reduced potential to produce EPS.[2]

From this series 8-[3-[bis(4-fluorophenyl)amino]propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**3**) was



selected for further pharmacological evaluation. Because of its unique pharmacological profile, we chose to synthesize a series of analogues with various substituents on the triazaspirodecanone moiety and evaluate these derivatives in several test models.

Chemistry. The general routes used for synthesis of the target compounds are outlined in Scheme I. The preparation of **3** was described previously.[1] The substituted phenyl compounds **5–11** were synthesized by the same general route used to prepare **3**, i.e. alkylation of the appropriately substituted phenyltriazaspirodecanone with *N*-(3-chloropropyl)-4-fluoro-*N*-(4-flurophenyl)benzenamine (**1**) (method A). The 4-(4-chlorophenyl) analogue **4** was synthesized in four steps from 1-[3-[bis(4-fluorophenyl)-amino]propyl]piperidin-4-one (**2**) by the general route for the synthesis of phenyltriazaspirodecanones described by Janssen (method B).[3] The substituted piperidone was prepared by alkylation of 4-piperidone with **1**. The N-3-alkylated analogues, **11–17**, **21**, and **22**, were synthesized from the appropriate amide and alkyl halide by traditional alkylation methods (method C). The 3-(hydroxymethyl) compound, **18**, was prepared by treatment of **3** with aqueous formaldehyde. Compound **18** was subsequently converted to the 3-[(acetoxy)methyl] and 3-[(phenylmethoxy)methyl] derivatives, **19** and **20**, respectively.

Results and Discussion

The target compounds were tested for their ability to inhibit [^{3}H]haloperidol binding to dopamine receptors in striatal homogenates at 10^{-8} M concentration.[4] They were evaluated for potential antipsychotic activity in mice by two-part test designed to measure inhibition of spontaneous locomotor activity (LMA) and impairment of motor function (falling off an inverted screen.)[5] This test is based on the observation that known antipsychotic agents inhibit spontaneous locomotion in mice at doses that do not cause impaired motor function. Compounds that inhibited binding ≥60% at 10^{-8} M and had a minimally active dose of ≤30 mg/kg in the inhibition of the LMA/screen falloff test were evaluated in secondary test models. Test results are listed in Table I.

Additional assessments of compounds showing activity in the initial screens were obtained by measuring the suppression of high base line self-stimulation with electrodes placed in the medial forebrain bundle of the posterior hypothalamus of male hooded rats.[6] ED_{50}'s were calculated for compounds with activity in this model.

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(1) Wise, L. D.; Pattison, I. C.; Butler, D. E.; Lewis, E. P.; Lobbestael, S. J.; Nordin, I. C.; Poschel, B. P. H; Coughenour, L. L. *J. Med. Chem.* **1985**, *28*, 606.
(2) Tamminga, C. "Neuroleptics: Neurochemical, Biochemical, and Clinical Perspectives"; Coyle, J. T., Enna, S. J., Eds.; Raven Press: New York, 1983; p 281.
(3) Janssen, P. A. J. U.S. Patent, 3 155 670, 1964; *Chem. Abstr.* **1965**, *62*, 7770. Janssen, P. A. J. U.S. Patent, 3 238 216, 1966; *Chem. Abstr.* **1966**, *65*, 8922.
(4) Burt, D. R.; Greese, I.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 800.
(5) Coughenour, L. L.; McLean, J. R.; Parker, R. B. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351.
(6) Olds, J.; Miller, P. J. *Comp. Physiol. Psychol.* **1954**, *47*, 419.

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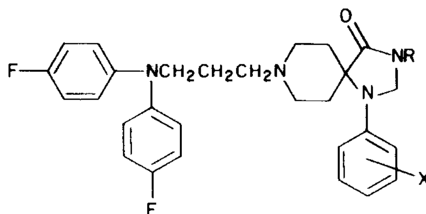
Table I. 8-[3-[Bis(4-fluorophenyl)amino]propyl]-1-aryl-1,3,8-triazaspiro[4.5]decan-4-ones



no.	X	R	method[a]	recryst solvent	yield,[b] %	mp, °C	formula[c]	anal.[d]	inhibn [^{3}H]haloperidol binding,[e] % (10^{-8} M)	LMA-inverted screen test[f] MED, mg/kg, ip	supp of self-stim[g] ED_{50}, mg/kg, po	rat catalepsy,[h] ED_{50}, mg/kg po
3	H	H	A	EtOAc	79	155–156	$C_{28}H_{30}F_2N_4O$	C, H, N	96	5	1.65 (0.9)	81
4	4-Cl	H	B	EtOAc	45	165–167	$C_{28}H_{29}ClF_2N_4O$	C, H, N	80	30	2.0	
5	4-OCH$_3$	H	A	EtOAc	41	145.5–146	$C_{29}H_{34}F_2N_4O_2$	C, H, N	58	30	>16	
6	4-CH$_3$	H	A	EtOAc	47	126–146	$C_{29}H_{32}F_2N_4O$	C, H, N	89	≤10	>16	
7	4-CH(CH$_3$)$_2$	H	A	EtOAc	55	151.5–153	$C_{31}H_{36}F_2N_4O$	C, H, N	41	>100		
8	3-CF$_3$	H	A	EtOAc	52	160.5–162.5	$C_{29}H_{29}F_5N_4O$	C, H, N	88	>100		
9	4-F	H	A	EtOAc	62	161.5–163	$C_{28}H_{29}F_3N_4O$	C, H, N	95	≤10	0.55	4.5
10	3-F	H	A	EtOAc	54	161.5–164	$C_{28}H_{29}F_3N_4O$	C, H, N	82	30	0.78	4.1
11	2-F	H	A	EtOAc	50	148–150	$C_{28}H_{29}F_3N_4O$	C, H, N	54	...	>16	
12	H	CH$_3$	C	EtOH	46	230–231	$C_{29}H_{32}F_2N_4O{\cdot}HI$	C, H, N	72	≤10	2.84	33
13	4-F	CH$_3$	C	MeOH	61	187–188.5	$C_{29}H_{31}F_3N_4O{\cdot}C_4H_4O_4$	C, H, N	91	≤10	0.96	54
14	3-CF$_3$	CH$_3$	C	MeOH	28	251–254	$C_{29}H_{31}F_5N_4O{\cdot}HCl$	C, H, N	76	30	>16	
15	H	CH$_2$CH=CH$_2$	C	*i*-PrOH	43	215–216	$C_{31}H_{34}F_2N_4O{\cdot}HCl$	C, H, N	92	≤10	1.06	22
16	H	CH$_2$–◁	C	EtOH	25	117–119	$C_{32}H_{36}F_2N_4O$	C, H, N	76	30	8.39	200
17	H	CH$_2$Ph	C	EtOH	51	132–133	$C_{35}H_{36}F_2N_4O$	C, H, N	76	>100		
18	H	CH$_2$OH	D	*i*-PrOH	80	286–287	$C_{29}H_{32}F_2N_4O_2{\cdot}HCl$	C, H, N	65	≤10	0.89	11
19	H	CH$_2$OAc	E	MeOH	51	181.5–183	$C_{31}H_{34}F_2N_4O_3{\cdot}C_4H_4O_4$	C, H, N	94	≤10	1.03	17
20	H	CH$_2$OCH$_2$Ph	C	MeOH	33	163–165	$C_{36}H_{38}F_2N_4O_2{\cdot}C_4H_4O_4$	C, H, N	85	100	>16	
21	H	CH$_2$CH$_2$NEt$_2$	C	*i*-PrOH	74	115–118	$C_{34}H_{43}F_2N_5O{\cdot}2HCl{\cdot}H_2O$	C, H, N	86	≤10	5.65	138

22	H	$CH_2CH_2CH_2NMe_2$	C	pet. ether	73–75	$C_{33}H_{44}F_2N_5O$	C, H,[i] N	72	30	16	200
23	spiperone							96	≤10	1.28	10.8
24	haloperidol							100[j]	0.30	0.14	0.10
25	clozapine							25[k]	10	14.3	133

[a] See the Experimental Section for general preparative procedures. [b] No attempts were made to maximize yields. [c] Compounds were characterized as free bases except where salts are noted below. [d] Except where noted, values obtained agreed with calculated values within 0.4%. [e] [^{3}H]Haloperidol binding to rat striatal membranes using 0.6 nM ligand was performed by the method of Burt et al.[3] Entries are one experiment done in triplicate. [f] With the exceptions of 1, 22, and 23 all compounds were evaluated at 10, 30, and 100 mg/kg. Each dose was replicated three times. The minimal effective dose (MED) was defined as being the lowest dose showing greater than 60% inhibition of locomotor activity with less than 60% screen falloff. [g] ED_{50}'s were obtained by linear regression from one dose higher and one dose lower than the ED_{50} value.[12] Correlation coefficients in the parentheses are shown for compounds that were tested at more than two doses. [h] At least three doses of compounds were administered and eight animals were used at each dose. ED_{50}'s were obtained by a nonlinear regression analysis.[12] [i] H: calcd, 7.85; found, 7.14. [j] IC_{50} = 0.28 nM. [k] IC_{50} = 60.0 nM.

Compounds with good activity in this test model were evaluated in rats for their ability to induce catalepsy, an effect normally believed to be related to clinical extrapyramidal side effects.[7]

As shown previously (see Table I), compound **3** was active in the initial in vitro binding assay and also in the inhibition of the locomotor activity/screen fallout test in mice. Administered orally to rats, it had an ED_{50} of 1.65 mg/kg in the suppression of the high base line self-stimulation test and an ED_{50} of 81 mg/kg in the catalepsy model.

Compounds **4–11** were examined in order to determine the effects of substitution on the 1-phenyl ring of the triazaspirodecanone moiety. With the possible exception of **7**, these compounds appear to bind to the dopamine receptor in vitro sufficiently well to suggest in vivo activity. The fact that the 4-chloro analogue **4** is highly active while the 4-methoxy and 4-methyl analogues, **5** and **6**, respectively, are inactive orally in the suppression of the self-stimulation test could be due to differences in the lipophilicity of these compounds or problems related to metabolism. Differences in lipophilicity may also be reflected in the lack of in vivo activity seen with the 3-(trifluoromethyl) and 2-fluoro analogues, **8** and **11**, respectively. The 4-isopropyl analogue **7** is less active in the binding assay and is also inactive in vivo. Its reduced activity is likely due to steric limitations at the 4-position of the 1-phenyl moiety.

The effects of various substituents on the N-3 nitrogen atom of the triazaspirodecanone were also examined. While the *N*-methyl analogues of **3** and **9**, compounds **12** and **13**, respectively, are slightly less active in the test of suppression of self-stimulation, both show a good separation between those doses predictive of efficacy and those causing catalepsy. In fact, **13** shows an enhanced separation. The cyclopropyl analogue, **16**, although less active than **3**, also maintains this separation. Increasing the size of the substituent to a benzyl group, compound **17**, results in complete loss of activity. The hydroxymethyl and (acetyloxy)methyl analogues, **18** and **19**, respectively, retain the desired activity profile while the (benzyloxy)methyl compound, **20**, shows little activity. Finally, the (diethylamino)ethyl derivative, **21**, is more potent than its homologue, (dimethylamino)propyl, **22**.

The SAR data presented above for this series suggest that the activity of these compounds is sensitive to substitution on the 1-phenyl moiety. However, the activity is less sensitive to substitution on the N-3 nitrogen atom. Only when very large groups such as benzyl or (benzyloxy)methyl are added is activity abolished. In contrast to data presented previously on the effect of substitution on the (diarylamino)alkyl portion of the molecule, separation between doses necessary for behavioral activity and catalepsy is not affected by changes at these positions. It was previously found that the [bis(4-fluorophenyl)amino]propyl moiety appears to be optimal.[1]

On the basis of its overall chemical and initial pharmacological profile, **3** was chosen for further evaluation in more comprehensive test models. Pertinent results in secondary tests are summarized in Table II and compared with the standards spiperone, haloperidol, and clozapine. Like other known antipsychotic agents **3** is active in the

(7) Van Rossum, J. M.; Boissier, J. R.; Janssen, P. A. J.; Julou, L.; Loew, D. M.; Moller Nielsen, I.; Munvad, I.; Randrup, A.; Stille, G.; Tedeschi, D. H. "Pharmacology of Neuroleptic Drugs. Modern Problems of Pharmacopsychiatry (Neuroleptics)"; Bobon, J., Janssen, P. A. J., Eds.; S. Karger: Basel, 1970; p 23.

Scheme I

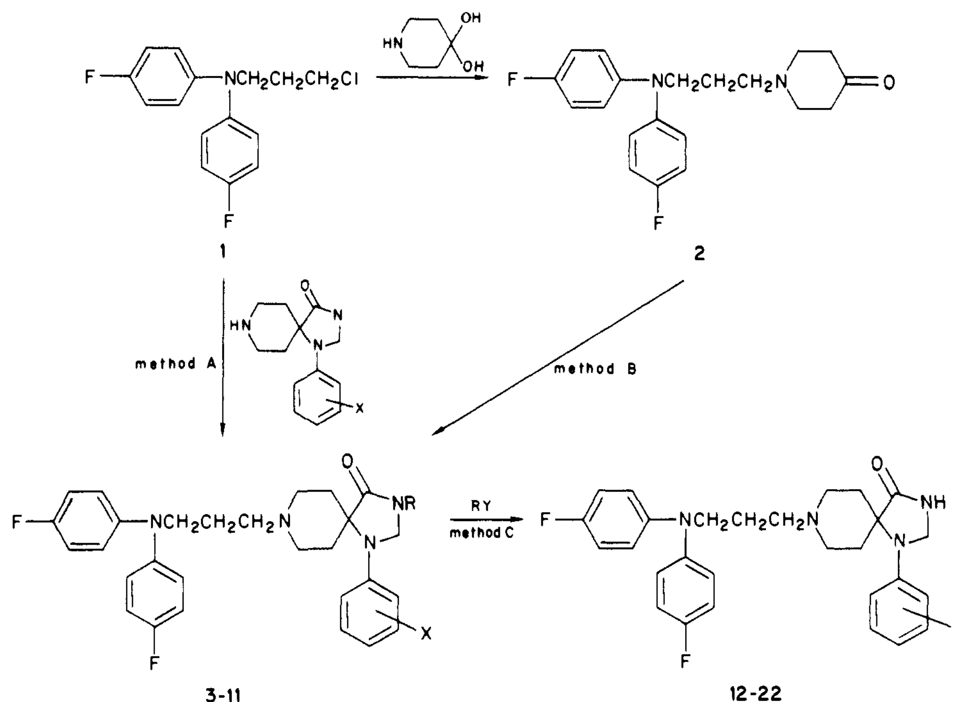


Table II. Effects of 3 vs. Haloperidol and Clozapine in Secondary Test Models[a]

test	3	haloperidol	clozapine
inhibn of Sidman avoidance in rats (ED_{50}, mg/kg po)[b]	2.8	0.3	14.4
catalepsy in rats (ED_{50}, mg/kg po)[c]	81.2	0.47	133
inhibn of Sidman avoidance in squirrel monkeys ED_{50}, mg/kg po)[b]	1.7	0.26	4.1
catalepsy in squirrel monkeys (ED_{50}, mg/kg po)[d]	30	2.0	>50
signs of EPS in cebus monkeys (MED, mg/kg po)[e]	2.5	0.30	>30
inhibn of [^{3}H]QNB binding (IC_{50}, nM)[f]	>100	54	48
inhibn of [^{3}H]WB-4101 binding (IC_{50}, nM)[g]	>1000	6000	20

[a] ED_{50}s were calculated by a nonlinear regression analysis.[12] [b] At least three doses of each drug were tested in four animals at each dose. [c] ED_{50}'s were measured from at least three doses of agent in eight animals at each dose level. [d] ED_{50}'s were calculated from at least three doses in four animals at each dose. [e] Minimal effective doses (MED) were measured in at least three animals. [f] Binding of [^{3}H]quinuclidinyl benzilate (QNB) to the muscarinic receptor was carried out as described by Ellis and Hoss.[9] [g] Binding of [^{3}H]-[[[(2,6-dimethoxyphenoxy)ethyl]amino]ethyl]-1,4-benzodioxane (WB4101) to the α_1-adrenergic receptor was carried out by the method of UPrichard et al.[10]

Sidman avoidance test in rats (ED_{50} = 2.8 mg/kg po) and in squirrel monkeys (ED_{50} = 1.7 mg/kg po).[8] In the squirrel monkey as in the rat, 3 shows quite a large separation between doses in tests predictive of activity and those that cause catalepsy. All clinically available antipsychotic agents have a profile typified by haloperidol; that is, they cause catalepsy at doses that are quite similar to those that are effective in behavioral tests predictive of antipsychotic activity. In contrast, clozapine, a drug that is claimed to be devoid of EPS, shows a relatively large separation in these test models. This again suggests that 3 might have a profile more like clozapine and not produce neurological side effects normally seen with available antipsychotic drugs.

However, in an alternative EPS test model in which cebus monkeys were sensitized to haloperidol, 3 showed signs of EPS at doses very similar to doses necessary for activity in the aforementioned predictiors of efficacy (MED = 2.5 mg/kg po).[9] This lack of separation is quite similar to that seen with haloperidol and other clinically available antipsychotic drugs. Clozapine, on the other hand, does not show signs of EPS in this model within the dose range tested.

It has been suggested that clozapine's unique profile may be due to its anticholinergic and/or antiadrenergic effects. Compound 3 was examined in the muscarinic cholinergic and α_1-adrenergic binding assays.[10,11] Unlike clozapine it did not bind to any of these receptors in vitro at reasonable concentrations. The lack of adrenergic binding would also suggest that 3 should be free of the cardiovascular side effects observed with many antipsychotic agents.

The results obtained in the catalepsy model suggest that 3 may have a reduced liability for EPS compared with haloperidol and other similar drugs. Data from the haloperidol-sensitized cebus monkey model on the other hand predict that 3 should have a profile similar to haloperidol and most other clinically available antipsychotic drugs that cause EPS at their therapeutic doses. Only the clinical evaluation of 3 will ultimately determine its liability producing for such side effects.

Experimental Section

Melting points were determined in a Thomas-Hoover melting point apparatus in open capillary tubes. The structures of the compounds were confirmed by elemental analysis, infrared spectrometry, and NMR spectrometry. Infrared spectra were recorded on a Digilab FTP-14 infrared spectrometer, and NMR

(8) Sidman, M. *J. Comp. Physiol. Psychol.* **1953**, *46*, 253.

(9) Barany, S.; Ingvast, A.; Gunne, L. M. *Res. Commun Pathol. Pharmacol.* **1979**, *25*, 269.

(10) Ellis, J.; Hoss, W. *Brain Res.* **1980**, *193*, 189.

(11) U'Prichard, D. C.; Bechtel, W. D.; Rouot, B. M.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 47.

spectra were obtained either on a Varian EM 390 or Brucker WH 90 spectrometer and were consistent with the proposed structures. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values. TLC was carried out with 0.25-mm silica gel 60 F254 (E. Merck) glass plates. GLC was carried out in a Schimadzu GC Mini 2 or a Perkin-Elmer Model 910 gas chromatograph equipped with FID.

Substituted 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-ones. The synthesis of 1-(4-fluorophenyl)-, 1-(4-methoxyphenyl)-, and 1-(4-methylphenyl)-1,3,8-triazaspiro[4.5]decan-4-ones are described by Janssen.[3] The following analogues were prepared by procedures similar to those outlined previously:[3] 1-(2-fluorophenyl)-, 1-(3-fluorophenyl)-, 1-[4-(1-methylethyl)phenyl]-, and 1-[3-(trifluoromethyl)phenyl]-1,3,8-triazaspiro[4.5]decan-4-one. These amines were not isolated but were alkylated directly as described in method A.

Method A. 8-[3-[Bis(4-fluorophenyl)amino]propyl]-1-(4-fluorophenyl)-1,3,8-triazaspiro[4.5]decan-4-one (9). A mixture of 14.4 g (0.051 mol) of *N*-(3-chloropropyl)-4-fluoro-*N*-(4-fluorophenyl)benzenamine (1),[1] 12.8 g (0.051 mol) of 1-(4-fluorophenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 8.98 g (0.051 mol) of potassium carbonate, and 0.5 g (3.3 mmol) of sodium iodide in 150 mL of 4-methyl-2-pentanone was refluxed for 16 h. The solvent was evaporated, and the residue was partitioned between chloroform and water. The organic extracts were dried over magnesium sulfate and evaporated to give 15.7 g (62%) of **9**, mp 159–161 °C. Anal. ($C_{28}H_{29}F_3N_4O$) C, H, N.

Method B. 8-[3-[Bis(4-fluorophenyl)amino]propyl]-1-(4-chlorophenyl)-1,3,8-triazaspiro[4.5]decan-4-one (4). A mixture of 30 g (0.10 mol) of 1, 17 g (0.11 mol) of 4-piperidinone hydrochloride hydrate, 21 g (0.25 mol) of sodium carbonate, and 10 g of sodium iodide in 400 mL of DMF was stirred at 75–90 °C for 20 h. The reaction mixture was filtered, and the solvent was evaporated. The residue was dissolved in dichloromethane, washed with water, and dried over magnesium sulfate. Evaporation of the solvent afforded 36 g of oil. The oil was chromatographed on a silica gel column (ethyl acetate). There was obtained 23 g (67%) of 1-[3-[bis(4-fluorophenyl)amino]-propyl]-4-piperidinone (2) characterized as a (*Z*)-2-butenedioate salt, mp 121–124 °C. Anal. ($C_{20}H_{22}F_2N_2O{\cdot}C_4H_4O_4$) C, H, N.

A mixture of 22 g (0.065 mol) of **2** and 10.1 g (0.07 mol) of 4-chlorobenzenamine in 150 mL of toluene was refluxed for 2.5 h with a Dean–Stark trap. After the mixture was cooled to 50 °C, 15 g (0.18 mol) of acetone cyanohydrin was added. The acetone was slowly distilled. The solvent was evaporated in vacuo, and the residue was chromatographed on a silica gel column (ethyl acetate). The product was converted to a (*Z*)-2-butenedioate salt in ethanol. There was obtained 22 g (57%) of 1-[3-[bis(4-fluorophenyl)amino]propyl]-4-[(4-chlorophenyl)amino]-4-piperidinecarbonitrile, (*Z*)-2-butenedioate salt (1:1), mp 175–177 °C. Anal. ($C_{27}H_{27}ClF_2N_4{\cdot}C_4H_4O_4$) C, H, N.

A solution of 18 g (0.038 mol) of 1-[3-[bis(4-fluorophenyl)-amino]propyl]-4-[(4-chlorophenyl)amino]-4-piperidinecarbonitrile in 170 mL of concentrated sulfuric acid was allowed to stand at 25 °C for 24 h. The solution was poured into excess dilute ammonium hydroxide and ice. The aqueous mixture was extracted with dichloromethane. The organic extracts were washed with saturated sodium bicarbonate solution and dried over magnesium sulfate. Evaporation of the solvent afforded 16 g (85%) of solid, mp 154–156 °C. Recrystallization from ethyl acetate gave pure 1-[3-[bis(4-fluorophenyl)amino]propyl]-4-[(4-chlorophenyl)-amino]-4-piperidinecarboxamide, mp 157–159 °C. Anal. ($C_{27}H_{29}ClF_2N_4O$) C, H, N.

To 5.0 g (0.01 mol) of 1-[3-[bis(4-fluorophenyl)amino]-propyl]-4-[(4-chlorophenyl)amino]-4-piperidinecarboxamide in 120 mL of hot toluene was added 10 mL of dimethoxy-*N*,*N*-dimethylmethanamine. The solution was refluxed for 48 h and concentrated. The residue was treated with (*Z*)-2-butenedioic acid in ethanol to yield 3.5 g (56%) of 8-[3-[bis(4-fluorophenyl)amino]propyl]-1-(4-chlorophenyl)-1,3,8-triazaspiro[4.5]-dec-2-en-4-one, (*Z*)-2-butenedioate salt, mp 207–209 °C. Anal. ($C_{28}H_{27}F_2N_4O{\cdot}C_4H_4O_4$) C, H, N.

A solution of 2.4 g (4.7 mmol) of 8-[3-[bis(4-fluorophenyl)-amino]propyl]-1-(4-chlorophenyl)-1,3,8-triazaspiro[4.5]dec-2-en-4-one in 50 mL of methanol was hydrogenated at 50 psi over a catalytic amount of 5% Pt/C. The suspension was filtered, and the filtrate was concentrated. The residue was recrystallized from ethyl acetate to give 1.2 g (50%) of *4*, mp 165–167 °C. Anal. ($C_{28}H_{29}F_2ClN_4$) C, H, N.

Method C. 8-[3-[Bis(4-fluorophenyl)amino]propyl]-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Monohydriodide (12). To a solution of 7.15 g (15 mmol) of **3** in 50 mL of DMF was added a suspension of 0.41 g (18 mmol) of sodium hydride (obtained by washing 0.82 g of a 50% sodium hydride–mineral oil suspension with toluene) portionwise in toluene. After hydrogen evolution had ceased, 2.13 g (15 mmol) of methyl iodide was added dropwise, and the mixture was stirred at room temperature for 10 h. The solvent was removed in vacuo, and the residue was purified by chromatography on a silica gel column (dichloromethane–methanol (95:5)). There was obtained 4.28 g (46%) of **12**, mp 230–231 °C. Anal. ($C_{29}H_{32}F_2N_4O{\cdot}HI$) C, H, N.

Method D. 8-[3-[Bis(4-fluorophenyl)amino]propyl]-3-(hydroxymethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Monohydrochloride (18). A mixture of 11.9 g (0.025 mol) of **3** and 12.8 g (0.16 mol) of 37% aqueous formaldehyde in 100 mL of 2-propanol was refluxed for 53 h. The mixture was evaporated, and the viscous residue was treated with 2-propanolic hydrogen chloride. The resulting solid was collected and recrystallized from 2-propanol to yield 10.9 g (80%) of **18**, mp 286–287 °C. Anal. ($C_{29}H_{32}F_2N_4O_2{\cdot}HCl$) C, H, N.

Method E. 3-[(Acetyloxy)methyl]-8-[3-[bis(4-fluorophenyl)amino]propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, (*Z*)-2-Butenedioate Salt (1:1) (19). A mixture of 15.2 g (0.03 mol) of **18**, 8.20 g (0.10 mol) of sodium acetate, and 20.4 g (0.20 mol) of acetic anhydride was refluxed for 2 h. The reaction mixture was poured into water and extracted with dichloromethane. The organic extracts were dried over magnesium sulfate and evaporated. The residue was treated with (*Z*)-2-butenedioic acid in methanol. The resulting precipitate was collected and recrystallized from methanol. There was obtained 10.2 g (51%) of **19**, mp 181.5–183 °C. Anal. ($C_{31}H_{34}F_2N_4O_3{\cdot}C_4H_4O_4$) C, H, N.

Pharmacological Methods

[^{3}H]Haloperidol Receptor Binding Assay.[4] The relative affinities of compounds for dopamine receptors were evaluated on the basis of their ability to displace [^{3}H]haloperidol from striatal membranes prepared from Long–Evans hooded rats. Rats were decapitated, the brains were removed, and the corpus striata were dissected. The corpus striata were homogenized in 40 volumes of 50 mM Tris–HCl buffer (pH 7.6) and centrifuged. Pellets were rehomogenized in 50 volumes of the same buffer and used for the binding assay. Incubations were carried out in 10 mL of 50 mM Tris-HCl buffer (pH 7.6) containing 2 mg/mL of original wet tissue weight of homogenate, 100 μL of test agent or solvent, and 0.6 nM of [^{3}H]haloperidol. Nonspecific binding was determined in the presence of 0.1 μM (+)-butaclamol. Samples were incubated at 25 °C for 40 min. Incubation was terminated by rapid filtration under reduced pressure through glass fiber filters (Whatman GF/B). The filters were rinsed three times with 10 mL of Tris-HCl buffer. The filters were placed in 10 mL of scintillation cocktail (Beckman Ready-Solv HP) and shaken for 1 h. Radioactivity retained on the filter was determined by liquid scintillation spectrophotometry. Compounds were initially evaluated at 10 nM. IC_{50}'s when determined were calculated from a nonlinear computer curve fit of the data from four or more concentrations, done in triplicate.[12]

Inhibition of Locomotion–Screen Falloff Test.[5] Nine unfasted Swiss–Webster male mice (Buckberg Laboratories) weighing 20–30 g were tested at each dose of drug. Treatments were administered intraperitoneally 1 h prior to testing. All dosages were calculated as parent

(12) Parker, R. B.; Waud, D. R. *J. Pharmacol. Exp. Ther.* **1973**, *183*, 1.

compound and given in volumes of 10 mL/kg. Compounds were dissolved or suspended in 0.2% methocel. Control animals were injected with methocel. A two-part testing procedure was started 1 h post-injection. First, the screen test was performed. This test consisted of placing mice on individual wire screens that were rotated 180° at the start of a 60-s observation period. The number of mice falling off the inverted screen was recorded. Immediately following the screen test, the animals were tested for inhibition of locomotion. Locomotor activity was measured in actophotometer chambers, three mice per chamber. The actophotometer consisted of a cylindrical chamber whose center contained the illumination for six photocells located on the perimeter of the chambers. Six light beam interruptions equaled one count. Locomotion activity was recorded by computer at 10-min intervals for 60 min. Results obtained from the inverted screen test were expressed as the percent of mice falling off the screen. Data derived from the locomotor activity of the drug-treated mice were compared to the activity of vehicle-treated animals and were expressed as percent inhibition of spontaneous locomotion. All percentages for inhibition of locomotion were based upon data accumulated for 1 h. Both phases of testing were graded: A = 60–100%; C = 31–59%; N = 0–30%. An overall rating of A resulted from an A rating in inhibition of locomotion and either a C or N rating in screen falloff. A C rating resulted from an A rating in both or a C rating in locomotion and a C or N rating in the screen portion. All other combinations resulted in an N rating.

Suppression of High Base Line Self-Stimulation.[6] Adult male hooded (Long–Evans) rats were implanted with permanent electrodes in the medial forebrain bundle of the posterior hypothalamus. After the animals recovered from surgery, they were trained in a Skinner box to press a lever to stimulate their own brains electrically (40 μA, 0.4 s/lever press). The rapid response rates generated by these conditions served as behavioral base lines. Compounds were administered orally. During all tests the self-stimulation behavior of the animals was continuously recorded graphically on cumulative recorders. A compound was considered active if the base line rates of self-stimulation were reduced by 50% for 1.5 h or more by the agent. Four rats were run for each dose level; ED_{50} dose levels were calculated by computer analysis.[12]

Rat Catalepsy Test.[7] Fasted male Long–Evans hooded rats (180–220 g) were used one time for each compound tested. Drugs were administered by oral intubation, and if insoluble, the compounds were suspended with 0.2% methylcellulose. At least three doses of the agent were administered and eight animals were used for each dose level. The rats were tested every 30 min for the first hour and then hourly for 6 h. If necessary, a 24-h reading was made. The animals were tested for catalepsy by placing their forepaws on a horizontal metal rod 11.5 cm above the table top and their hind paws on the table top. An animal was considered fully cataleptic (scored as 1.0) if it maintained this abnormal position on the bar for 30 s. Partial catalepsy (scored 0.5) resulted when a rat remained on the rod from 20–29 s. A minimum of three attempts was made to obtain catalepsy. The maximum number of rats considered cataleptic at any one time for each dose was used to determine the ED_{50}. Partial catalepsy scores were summed and rounded off to the highest whole number. The ED_{50}'s were obtained by a nonlinear regression analysis.[12]

Catalepsy in Squirrel Monkeys.[7] Adult squirrel monkeys (600–1000 g) were used with at least 1 week between dosings. Subjects were fasted overnight and, after dosing, were observed at hourly intervals for at least 6 h and at 24 h. Catalepsy was judged present if a subject could be placed in an abnormal position (i.e., hanging upside down on a vertical chain-link screen) for at least 1 min. Subjects were captured by hand and restrained gently until struggling was minimal. The tester held the animal by the neck and hips, inverted the monkey, and placed it against the vertical chain-link screen. When it was certain that the monkey was supporting its own weight, the animal was gently released and timing was begun. For each observation a maximum of three attempts was made to obtain catalepsy. Catalepsy was judged on an all-or-none basis, and it was only necessary for a subject to meet the criteria for one observation. Four animals were used at each dose, and at least three doses were examined. ED_{50}'s were determined by using a nonlinear regression technique for quantal data.[12]

Sidman Avoidance Procedures.[8] Mature male Long–Evans rats or squirrel monkeys were trained on a modified Sidman avoidance schedule with standard operant conditioning chambers equipped with a single wall-mounted response lever. Depression of the lever postponed the delivery of electric shock through the grid floor (2.4 mA/0.5 s for rats and 4.0 mA/0.5 s for monkeys) for 20 s (R–S interval = 20 s). Failure to depress the lever resulted in the delivery of shock every 10 s (S–S interval = 10 s). Depression of the lever at any time during the R–S interval reset the R–S interval to 20 s. Once the S–S interval was initiated, only a response during the 0.5-s shock delivery could reset the conditions to the start of the R–S interval. The duration of each test was 6 h. Drug effects were expressed as percentage inhibition of nondrug avoidance responding, with each animal serving as its own control. At least three doses of each drug were tested in four animals, with test sessions separated by at least 1 week. ED_{50}'s for avoidance inhibition were calculated from nonlinear regression analysis of the dose–effect functions.[12]

Extrapyramidal Side Effect Test in Cebus Monkeys.[9] Cebus monkeys were chronically treated with 2 mg/kg of haloperidol po once a week. After 6–12 weeks of treatment the monkeys became sensitized to haloperidol and related drugs whose effects are mediated by dopaminergic blockade. Once sensitized, the monkeys always developed dystonias with low (0.5 mg/kg) doses of haloperidol. The test procedure consisted of dosing (by gavage) the sensitized monkeys with test compound and observing them during the day for dystonias. Specific signs included involuntary twisting of the neck or torso, tongue protrusion, compulsive biting, oral dyskinesias, flailing about the cage, tonic extension of the limbs, and bizarre hand postures. Monkeys showing any number or degree of unequivocal signs of dystonias were identified as positive responders at that dose. Minimal effective doses for dystonic effects were determined.

Muscarinic Anticholinergic Receptor Binding Assay.[10] The relative affinities of compounds for the muscarinic cholinergic receptor were evaluated by their ability to displace [^{3}H]quinuclidinyl benzilate. Male Long–Evans rats (150–200 g) were decapitated. Brain tissues (minus the cerebellum and brain stem) were removed and homogenized in 10 volumes of ice-cold 0.32 M sucrose and centrifuged at 1100*g*. The supernatant was decanted and stored on ice until use. Incubations were carried out on triplicate samples containing 0.6 nM of [^{3}H]QNB, 0.5 mg/mL of original wet tissue weight of brain membranes, and 100 μL of various concentrations of test agent in a final incubation volume of 10 mL of 50 nM $NaKPO_4$ buffer at

pH 7.4. Samples were incubated for 1 h at 25 °C. Incubations were terminated by rapid filtration under reduced pressure through glass fiber filters (Whatman GF/B). The filters were rinsed three times with 10-mL aliquots of cold assay buffer. The radioactivity retained on the filters was counted on a liquid scintillation spectrophotometer in 10 mL of Beckman H-P scintillation cocktail after being mechanically shaken for 1 h. Nonspecific binding, which was defined as the binding in the presence of 100 μM oxotremorine, was subtracted from the mean of the triplicate samples to determine specific binding. The concentration of test agent needed to displace 50% of the specific binding (IC_{50}) was determined by a nonlinear computer curve fit from four or more concentrations (in triplicate) of the test agent.[12]

α_1-Adrenergic Receptor Binding.[11] The relative affinities of compounds for the α_1-adrenergic receptor were evaluated on the basis of their ability to displace [^{3}H]WB-4101 from rat frontal cortex membranes. Male Long–Evans rats were decapitated, the brains were removed, and the frontal cortex membranes were dissected. The cortex tissue was homogenized in 50 volumes of 50 mM Tris-HCl buffer at pH 7.7. The homogenate was centrifuged twice with rehomogenization of the intermediate pellet in 50 mL of fresh buffer. The final pellet was resuspended in 50 mM the buffer at pH 7.7 at a concentration of 20 mg/mL of original wet tissue. Incubation samples contained 1.0 mL (or 10 mg/mL of wet tissue weight) of brain membranes, 100 μL of various concentrations of test agents, and 0.5 nM of [^{3}H]WB-4101 in a final volume of 2 mL of 50 mM Tris-HCl buffer at pH 7.7. Samples were incubated for 30 min at 25 °C and rapidly filtered under reduced pressure through Whatman GF/B filters. The filters were rinsed three times with 5-mL aliquots of 50 mM assay buffer and shaken for 1 h in 10 mL of Beckman H-P scintillation cocktail. The radioactivity retained on the filters was counted by liquid scintillation spectrophotometry. Nonspecific binding was defined as the binding in the presence of 100 μM (−)-norepinephrine. This was subtracted from the mean of the triplicate samples to determine specific binding. The concentration of test agent needed to displace 50% of the specific binding (IC_{50}) was determined by nonlinear computer curve fit from four or more concentrations (in triplicate) of the test agent.[12]

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Registry No. 1, 80119-31-9; **2**, 97861-25-1; **2** (free base), 80119-57-9; **3**, 80120-14-5; **4**, 97861-00-2; **5**, 97861-01-3; **6**, 97861-02-4; **7**, 97861-03-5; **8**, 97861-04-6; **9**, 80119-32-0; **10**, 97861-05-7; 11, 97861-06-8; **12**, 97877-59-3; **12** (free base), 97861-07-9; **13**, 97861-09-1; **13** (free base), 97861-08-0; **14**, 97861-10-4; **14** (free base), 97861-11-5; **15**, 97861-12-6; **15** (free base), 97861-13-7; **16**, 97861-14-8; **17**, 97861-15-9; **18**, 97861-16-0; **18** (free base), 97861-17-1; **19**, 97861-19-3; **19** (free base), 97861-18-2; **20**, 97861-21-7; **20** (free base), 97861-20-6; **21**, 97861-22-8; **21** (free base), 97861-23-9; **22**, 97861-24-0; 1-(4-fluorophenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 58012-16-1; 4-piperidinone, 41661-47-6; 4-chlorobenzenamine, 106-47-8; acetone cyanohydrin, 75-86-5; 1-[3-[bis(4-fluorophenyl)amino]propyl]-4-[(4-chlorophenyl)amino]-4-piperidinecarbonitrile, 97861-26-2; 1-[3-[bis(4-fluorophenyl)amino]propyl]-4-[(4-chlorophenyl)amino]-4-piperidinecarbonitrile maleate, 97861-27-3; 1-[3-[bis(4-fluorophenyl)amino]propyl]-4-[(4-chlorophenyl)amino]-4-piperidinecarboxamide, 97861-28-4; dimethoxy-*N*,*N*-dimethylmethanamine, 4637-24-5; 8-[3-[bis(4-fluorophenyl)amino]propyl]-1-(4-chlorophenyl)-1,3,8-triazaspiro[4.5]dec-2-en-4-one, 97861-29-5; 8-[3-[bis(4-fluorophenyl)amino]propyl]-1-(4-chlorophenyl)-1,3,8-triazaspiro[4.5]dec-2-en-4-one maleate, 97861-30-8; 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, 1021-25-6; 1-(4-methoxyphenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 1027-69-6; 1-(4-methylphenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 1023-87-6; 1-(4-isopropylphenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 97861-31-9; 1-[3-(trifluoromethyl)phenyl]-1,3,8-triazaspiro[4.5]decan-4-one, 97861-32-0; 1-(3-fluorophenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 97861-33-1; 1-(2-fluorophenyl)-1,3,8-triazaspiro[4.5]decan-4-one, 97861-34-2.

3-Phenyl-1-indanamines. Potential Antidepressant Activity and Potent Inhibition of Dopamine, Norepinephrine, and Serotonin Uptake

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A series of 3-phenyl-1-indanamines was synthesized and tested for potential antidepressant activity and for inhibition of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) uptake. Trans isomers were generally potent inhibitors of DA, NE, and 5-HT uptake, while cis isomers preferentially inhibited the uptake of 5-HT. The affinity for the DA-uptake site was very dependent on the aromatic substitution pattern where highest potency was found for 3′,4′-dichloro substituted compounds (**45**). This substitution pattern also resulted in high affinity for the NE- and 5-HT-uptake sites, but potent 5-HT-uptake inhibiting activity could also be obtained with other substitution patterns. Only small amines could be accommodated at the 5-HT-uptake site while larger amines such as piperazine could be accommodated both at the DA- and NE-uptake sites. The observed structure–activity relationships were explained from the results of superimpositions of a trans (**45**) and cis (**72**) isomer with 5-HT and DA, respectively, in relation to a proposed three-point binding of the uptake inhibitors at the uptake sites. Finally, comparison of the structures of the 3-phenyl-1-indanamines with other newer bicyclic catecholamine- and/or serotonin-uptake inhibitors revealed common structural elements important for potent DA-, NE-, and/or 5-HT-uptake inhibition.

Traditionally, the mode of action of antidepressant agents has been explained as being a result of facilitation of norepinephrinergic and/or serotonergic transmission caused by inhibition of norepinephrine (NE) and/or 5-hydroxytryptamine (5-HT) uptake. However, a few years ago it was found that chronic treatment with tricyclic antidepressants such as amitriptyline or imipramine induced subsensitivity of presynaptic dopamine (DA) auto-

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