Troponoids. 7.[1] Chemistry and Dopamine Agonist Activity of Ciladopa and Related Aralkyltroponylpiperazines

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A series of *N*-aralkyltroponylpiperazine derivatives were synthesized and evaluated for dopaminergic activity in rats rendered hypokinetic by the bilateral injection of 6-hydroxydopamine (6-OHDA) into the anterolateral hypothalamus. Several members of the series were active, and a structure–activity relationship is presented. A few selected compounds were also evaluated with regard to their ability to induce contralateral rotational behavior in rats with a unilateral 6-OHDA-induced lesion of the nigrostriatal dopamine (DA) pathway and to suppress elevated serum prolactin levels. The compounds were compared to bromocriptine. Some of the more potent analogues were also assayed for their binding affinity to dopamine (DA) and α_1-adrenergic receptors. The results (a) established that the potency of some of the compounds were comparable or superior to that of bromocriptine, (b) indicated that potent dopaminergic activity was dependent on the presence of both a substituted phenyl and a troponylpiperazine moiety, and (c) confirmed that the dopaminergic activity depends on relative and absolute stereochemistry.

Dopaminergic agonists are a relatively new class of therapeutic agents that alleviate Parkinson's disease and hyperprolactinaemia-associated neuroendrocrine disorders in patients.[2] The beneficial therapeutic effect of these drugs is based on their dopamine (DA) mimicking activity, exerted upon the striatum and pituitary, respectively.

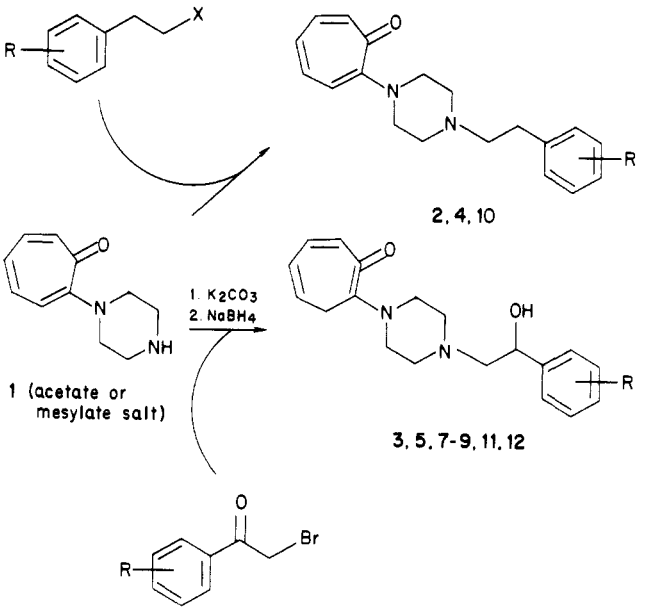
The present study describes the synthesis, as well as biochemical and pharmacological evaluation, of some *N*-aralkyltroponylpiperazine derivatives in animal models of parkinsonism and in experimentally induced hyperprolactinaemia. The compounds belong to a chemically novel class of DA receptor antagonists.[1]

Chemistry. In general the troponylpiperazine derivatives were prepared by condensation of troponylpiperazine salt **1**[1] with a suitable aralkyl halide (Scheme I). The derivatives **2**, **4**, and **10** were synthesized by condensation of appropriately substituted phenethyl bromide or phenethyl tosylate in aqueous or anhydrous acetonitrile in the presence of potassium carbonate. The desired bromides and tosylates were prepared from the corresponding alcohols in the conventional manner. Compounds **3**, **5**, **7–9**, **11**, and **12** were all prepared by condensation in acetonitrile of **1** with appropriate bromo ketones, in the presence of potassium carbonate, followed by reduction of the ketone with sodium borohydride. The catachol derivative **6** was obtained by demethylation of compound **4** with boron tribromide.

Compounds **20** and **21** (Table II) were synthesized by the condensation of *N*-troponylethylenediamine and *N*-troponylhomopiperazine, respectively, with 3,4-dimethoxyphenethyl bromide. The synthesis of **19** was achieved by condensation of *N*-phenylpiperazine with α-bromo-3,4-dimethoxyacetophenone, followed by the reduction of the carbonyl function with sodium borohydride. Compound **22** was synthesized by condensation of 3,4-dimethoxyphenethylamine with 2-methoxytropone.

The methylation of the carbon atom α to the nitrogen in a phenylethanolamine is known to produce significant changes in the biological profile. Compounds **13** and **14** were synthesized as described in Scheme II. The bromo ketone **23** was prepared as described[3] and was condensed in troponylpiperazine **1** in the usual manner. The resulting

Scheme I



ketone **24** was reduced with sodium borohydride to yield a mixture two diasterisomers (**13** and **14**). The NMR spectrum of **13** showed a carbinolic proton at δ 4.23 (J = 7.0 Hz) while **14** exhibited that at δ 4.85 (J = 4.2 Hz). It is reported[4] that the carbinolic proton of an erythro isomer appears at a higher chemical shift and has a smaller coupling constant than that of a threo isomer. On the basis of this evidence, compound **13** was assigned the threo and **14** the erythro stereochemistry (Scheme II).

It was observed that the major isomer (**13**) had dramatically lower biological activity, whereas the minor isomer (**14**) possessed potent dopamine agonist activity. A synthesis was devised to obtain the erythro isomer **14** in improved yield. This pathway is shown in Scheme III.

Bromo ketone **23** was condensed with benzylpiperazine to yield the desired ketone **25**. This was smoothly debenzylated with 10% Pd/C to yield the ketone **26**. Reduction of the carbonyl group of **26** with sodium borohydride yielded the alcohol **27**, which exhibited a carbinolic

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(1) Bagli, J.; Bogri, T.; Voith, K. *J. Med. Chem.* **1984**, *27*, 875.
(2) Parkes, D. *Adv. Drug. Res.* **1977**, *12*, 247.
(3) Lespagnol, A.; Cuingnet, E. *Ann. Pharm. Fr.* **1960**, *18*, 445.
(4) Tayalor, C. A., Jr.; Smith, H. E.; Goldberg, M. R.; Robertson, D. *J. Med. Chem.* **1981**, *24*, 1261.

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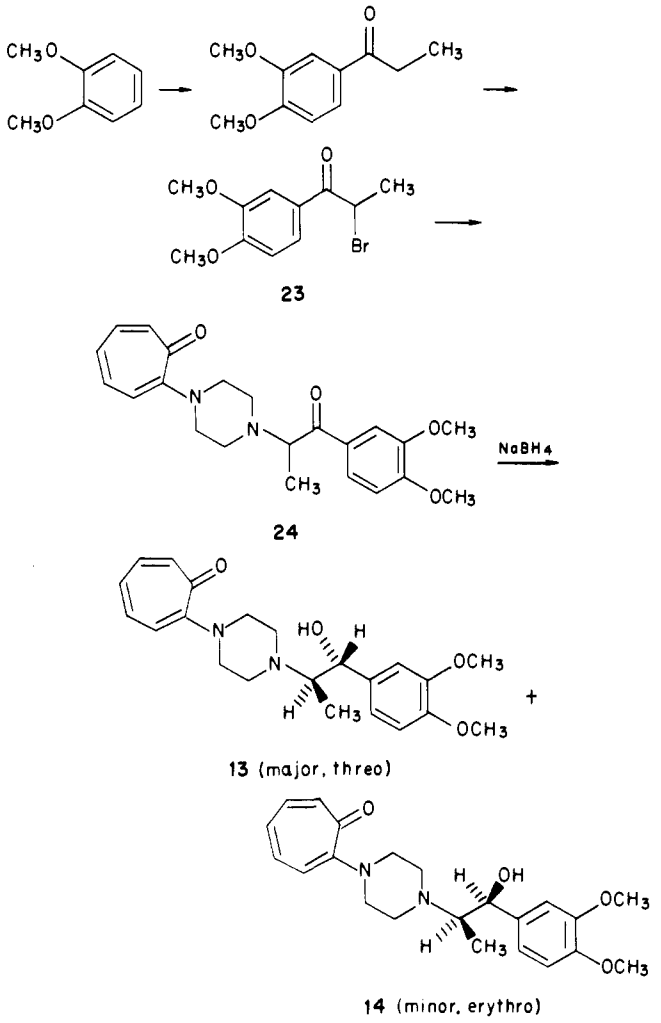
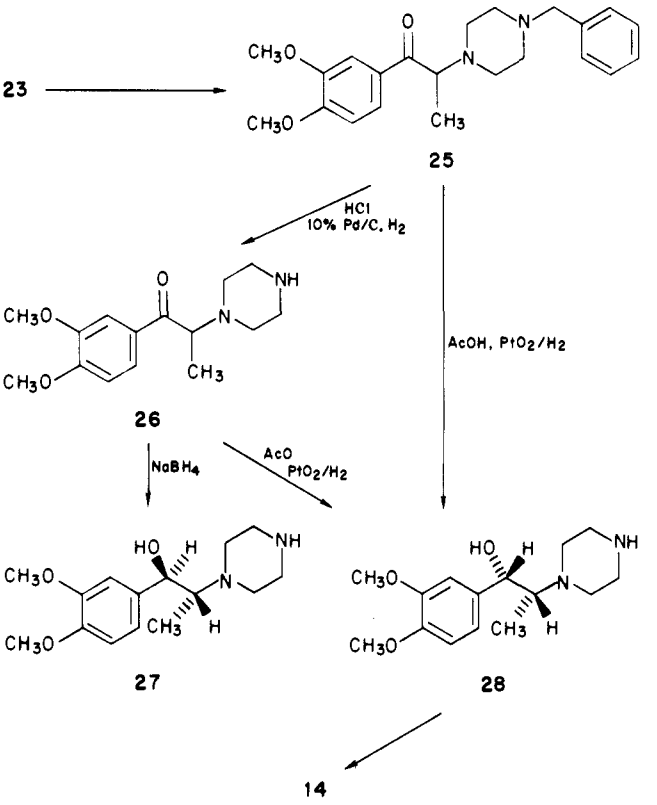
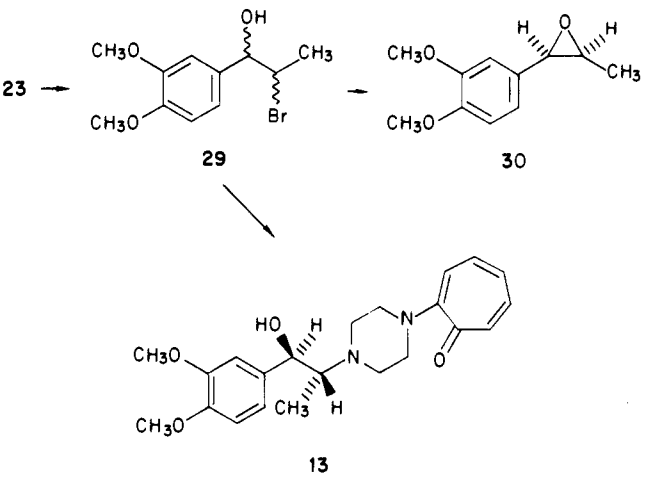
Scheme II



Scheme III



Scheme IV



proton at δ 4.17 (J = 9.75 Hz), indicating threo configuration.[4] In contrast, the reduction with acetic acid–platinum oxide yielded alcohol **28**, which showed a carbinolic proton at δ 4.77 (J = 4 Hz), suggesting erythro stereochemistry. Alcohol **28** upon condensation with 2-methoxytropone yielded the corresponding erythro derivatives **14**.

The reduction of bromo ketone **23**, with sodium borohydride, led to bromohydrin **29**. The stereochemical configuration of compound **29** could not be conclusively assigned due to the complex splitting pattern and the close proximity of the chemical shift of the carbinolic and HCBr protons in the NMR spectrum. The carbinolic proton however was located at δ 4.45 ppm. Treatment of alcohol **29** with troponylpiperazine mesylate (**1**) under alkaline condition led to the formation of threo alcohol **13**. When this reaction was monitored by TLC, the formation of an intermediate was detected (Scheme IV). The intermediate may well be the epoxide **30**. This hypothesis was supported as follows. The alcohol **29** was smoothly transformed with sodium and methanol to an epoxide **30**. The NMR spectrum exhibited a benzylic proton at δ 3.9 (J = 4 Hz) and the other carbinolic proton was located at δ 3.2. This suggested the cis stereochemistry[5] for the epoxide **30**. The R_f value of this epoxide was identical with that of the intermediate detected in the transformation of **29** → **13**.

Compounds **5** and **14** were resolved into their optical enantiomers by using *d*- and *l*-ditoluoyltartaric acid. The treatment of **5** with the *l* acid yielded a tartarate salt ($[\alpha]_D$ +45.8°). The corresponding base had a rotation of $[\alpha]_D$ +11.7°. This was transformed to its hydrochloride salt **15**, $[\alpha]_D$ −10.7°. Using the *d* acid **5** led to the corresponding enantiomeric hydrochloride **16** ($[\alpha]_D$ +10.7°). Compound **16** was found to be the biologically active antipode. It was therefore decided to determine the absolute configuration of alcohol **16**. It was converted to its *p*-bromobenzoyl ester and subjected to single-crystal X-ray determination.[6] The absolute configuration was found to be *S*. Thus, the absolute configuration of the asymmetric carbon in **15** follows as *R* (Scheme V). Resolution of compound **14** similarly yielded enantiomers **17** and **18**.

Results and Discussion

The aim of the present research was to evaluate the effects of aralkyltroponylpiperazine derivatives in animal

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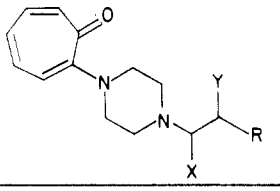
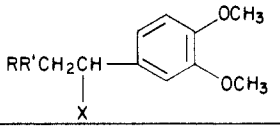
Table I. Effect of *N*-Aralkyltroponylpiperazines on 6-OHDA-Induced Hypokinesis in Rats

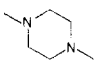
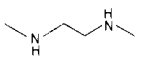
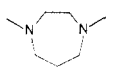


compd	X	Y	R	mp, °C	crystn solv[d]	formula (anal.)	dose, mg/kg sc	cumulative ambulation score, mean ± SEM
2	H	H	C_6H_5[e]	oil		$C_{19}H_{22}N_2O$	50	inactive
3	H	OH	C_6H_5	129–131	M/E	$C_{19}H_{22}N_2O_2$ (C, H, N)	50	inactive
4	H	H	3,4-$(OMe)_2C_6H_3$[a]	83–85	E/H	$C_{21}H_{26}N_2O_3$ (C, H, N)	10	101 ± 28 (8)[f]
5	H	OH	3,4-$(OMe)_2C_6H_3$[a]	122–124	M/E	$C_{21}H_{26}N_2O_4$·HCl (C, H, N)	10	191 ± 47 (10)
15	H	OH	3,4-$(OMe)_2C_6H_3$ (*R*(+))	123–125	C/H	$C_{21}H_{26}N_2O_4$ (C, H, N)	5	27 ± 15 (4)
16	H	OH	3,4-$(OMe)_2C_6H_3$ (*S*(−))[a]	122–124	C/H	$C_{21}H_{26}N_2O_4$ (C, H, N)	5	320 ± 83 (7)
6	H	H	3,4-$(OH)_2C_6H_3$[b]	256–258	M	$C_{19}H_{22}N_2O_3$·HBr (C, H, N)	25	98 ± 57 (7)
7	H	OH	4-$(OMe)C_6H_4$	139–140	Ea/H	$C_{20}H_{24}N_2O_3$ (C, H, N)	50	105 ± 65 (5)
8	H	OH	3-$(OMe)C_6H_4$	105–107	Ea	$C_{20}H_{24}N_2O_3$ (C, H, N)	50	inactive
9	H	OH	3,4,5-$(OMe)_3C_6H_2$	144–145	M/Ea	$C_{22}H_{28}N_2O_5$ (C, H, N)	50	inactive
10	H	H	4-F-C_6H_4[a]	208–210	M/E	$C_{19}H_{21}FN_2O$·HCl (C, H, N)	50	inactive
11	H	OH	4-F-C_6H_4	130–132	C/H	$C_{19}H_{21}FN_2O_2$ (C, H, N)	50	inactive
12	H	OH	3-OMe-4-OHC_6H_3	167–167.5	M	$C_{20}H_{24}N_2O_4$ (C, H, N)	25	inactive
13	CH_3	OH	3,4-$(OMe)_2C_6H_3$ (threo)[e]	133–135	M/E	$C_{22}H_{28}N_2O_4$	10	55 ± 16 (4)
14	CH_3	OH	3,4-$(OMe)_2C_6H_3$ (erythro)[c]	165–168	M/E	$C_{22}H_{28}N_2O_4$·$C_4H_4O_4$ (C, H, N)	2.5	111 ± 26 (4)
17	CH_3	OH	3,4-$(OMe)_2C_6H_3$ (erythro(+))[c]	161–163	M/E	$C_{22}H_{28}N_2O_4$·$C_4H_4O_4$ (C, H, N)	2.5	20 ± 8 (4)
18	CH_3	OH	3,4-$(OMe)_2C_6H_3$ (erythro(−))[c]	160–162	M/E	$C_{22}H_{28}N_2O_4$·$C_4H_4O_4$ (C, H, N)	1.25	186 ± 32 (11)
bromocriptine							10	112 ± 23 (12)

[a] Hydrochloride. [b] Hydrobromide. [c] Maleate. [d] M = methanol, E = ether, H = hexane, Ea = ethyl acetate, C = chloroform. [e] This compound was characterized by IR, UV, NMR, and MS. [f] Numbers in parentheses refer to number of rats in each group.

Table II. Effect of Structurally Modified Aralkyl Derivatives on 6-OHDA-Induced Hypokinesia in Rats



compd	R	R′	X	mp, °C	crystn solv[d]	formula (anal.)	dose, mg/kg sc	cumulative ambulation score, mean ± SEM
19	C_6H_5		OH	145–147	M	$C_{20}H_{26}N_2O_3$ (C, H, N)	50	inactive
20			H	80–82	M/E	$C_{19}H_{24}N_2O_3$[c]	50	inactive
21[a]			H	156–158	M/E	$C_{22}H_{28}N_2O_3$·2HCl[c]	50	inactive
22			H	110–112	Ea/H	$C_{17}H_{14}NO_3$ (C, H, N)	50	inactive

[a] Hydrochloride salt. [b] M = methanol, E = ether, Ea = ethyl acetate. [c] These compounds were characterized by IR, UV, NMR, and MS.

models of parkinsonism and in an experimentally induced hyperprolactinaemic state in order to determine their DA agonist activity.

We have evaluated 17 aralkyltroponylpiperazines, including the enantiomers of two of them, in the hypokinetic rat model,[7] many features of which mimic the parkinsonian syndrome.[1] The results are shown in Tables I and II and discussed in detail in the "structure–activity" section. While the previously described alkyltroponylpiperazines[1] were less active than bromocriptine on a milligram per

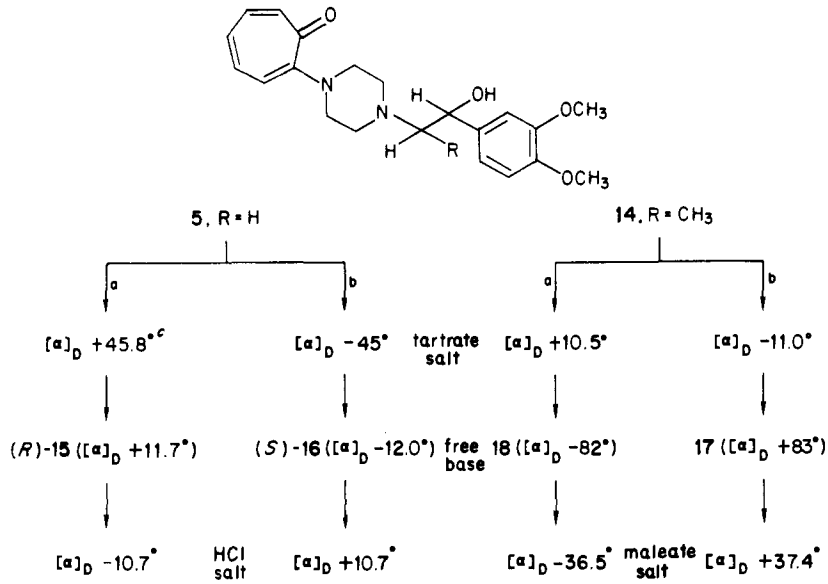
(7) (a) Ervin, G. N.; Fink, J. S.; Young, R. C.; Smith, G. P. *Brain Res.* **1977**, *132*, 507. (b) Smith, G. P.; Young, R. C. *Adv. Neurol.* **1974**, *5*, 427.

Table III. Rotational Behavior Induced by Selected Aralkyltroponylpiperazine Derivatives in Rats with a Unilateral 6-OHDA-Induced Lesion of the Nigro-striatal Dopamine Pathway

compd	dose, mg/kg sc	total no. of contralateral turns, mean ± SEM	max intensity, rotations/min, mean ± SEM
5	2.5	823 ± 110 (5)[a]	9.2 ± 1.1
15	2.5	140 ± 51 (6)	1.3 ± 0.5
16	2.5	1770 ± 330 (6)	10.7 ± 2.2
14	2.5	1903 ± 366 (6)	9.5 ± 1.4
17	2.5	121 ± 49 (6)	1.1 ± 0.5
18	2.5	2109 ± 490 (6)	14.6 ± 4.6
bromocriptine	4.0	836 ± 145 (4)	4.7 ± 1.4

[a] Numbers in parentheses refer to number of rats in each group.

Scheme V[a]



[a] (a) Ditoluyl-*l*-tartaric acid. (b) Ditoluyl-*d*-tartaric acid. (c) All rotations were recorded at 1% concentration in DMF at 25 °C.

Table IV. Effect of Selected Aralkyltroponylpiperazine Derivatives on Elevated Serum Prolactin Levels

	ED_{50},[a] mg/kg			ED_{50},[a] mg/kg	
compd	ip	po	compd	ip	po
5	0.48	2.00	18	0.04	0.15
16	0.36	0.90	17	2.20	
15	2.70		bromocriptine	0.24	0.60
14	0.20	0.25			

[a] ED_{50} is the dose that lowered the serum prolactin level to 50% of the treated control.

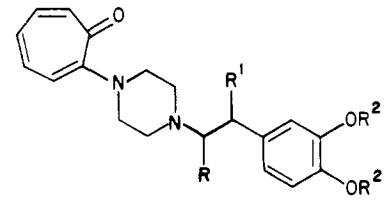
kilogram basis, several of the aralkyltroponylpiperazine derivatives were as potent as or more potent than bromocriptine.

Compounds **5** and **14** as well as their enantiomers were also evaluated in rats with a unilaterally degenerated nigrostriatal pathway and in rats with elevated prolactin levels.

DA agonists induce contralateral rotational behavior in unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats; this behavior is brought about by the development of postsynaptic DA receptor supersensitivity in the denervated striatum.[8] Table III illustrates that compounds **5** and **14** induced contralateral turning; the compounds were both more potent (regarding dose) and more efficacious (regarding intensity of turning) than bromocriptine. Results obtained in the rotating rat also established that only one of the enantiomers possessed DA agonist activity.

The effect of selected aralkyltroponylpiperazines on the serum prolactin level in the α-methyltyrosine-induced hyperprolactinaemic rats is summarized in Table IV. Compound **14** was more potent than compound **5**, supporting the notion that the incorporation of a methyl group α to the nitrogen enhances DA agonist activity. The observation that most of the pharmacological activity resides in only one of the enantiomers, i.e., in compounds **16** and **18**, was also confirmed in the hyperprolactinaemic rats. These results are in agreement with the ones seen in the hypokinetic and rotating rat models, confirming the correlation that exists between activity in animal models of

Table V. Effect of Some Aralkyltroponylpiperazines on Rat Striatal Dopaminergic Receptor Binding Displacement of [^{3}H]Haloperidol



compd	R	R^1	R^2	IC_{50},[a] μM
4	H	H	CH_3	0.88
6	H	H	H	0.76
5	H	OH	CH_3	1.50
13 (threo)	CH_3	OH	CH_3	8.55
14 (erythro)	CH_3	OH	CH_3	1.05
15 (*R*(+))	H	OH	CH_3	16.59
16 (*S*(−))	H	OH	CH_3	2.20
dopamine				1.20

[a] The values reported are the mean of at least two separate experiments.

parkinsonism and activity in an animal model of hyperprolactinaemia.

Multiple subtypes of DA receptors exist.[9] A classification scheme has been developed, such that subtypes of DA receptors whose effects involve activation of DA-sensitive adenylate cyclase are called D-1 receptors, whereas those not linked to the stimulation of the enzyme are called D-2 receptors.[10] More recently D-2 DA receptors have been shown to be linked to inhibition of adenylate cyclase.[11]

In this study the binding affinity of selected aralkyltroponylpiperazines for DA D-2 sites was assessed by examining their ability to displace [^{3}H]haloperidol from the rat striatal membranes.[12] The results obtained in this

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(9) Creese, I.; Hamblin, M. W.; Leff, S. E.; Sibley, D. R. In "Handbook of Psychopharmacology"; Iverson, L. L., Iverson, S. D., Snyder, S. H., Ed.; Plenum Press: New York, 1983; Vol. 17, p 81.
(10) Kebabian, J. W.; Calne, D. B. *Nature (London)* **1979**, *277*, 93.
(11) Munemura, M.; Cote, T. E.; Tsuruta, K.; Eskay, R. J.; Kebabian, J. W. *Endocrinology* **1980**, *107*, 1676.
(12) Burt, D. R.; Creese, I.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 800.

assay are shown in Table V. The nature of the substituents R, R', and R_α in the racemic compounds **4**, **5**, and **6** did not influence the binding appreciably, as all exhibited about equal affinities. The stereoisomer **14** (erythro) exhibited binding affinity about equal to that of dopamine. In contrast, the threo isomer had an 8-fold lesser affinity. The S enantiomer **16** and its racemic **5** exhibited about equal affinity for [^{3}H]haloperidol binding sites, being about equipotent to dopamine. In comparison the R-(+) enantiomer **15** had an 8-fold lesser affinity then its corresponding S-(−) enantiomer **16**.

To assess the activity of these compounds on D-1 DA receptors, the activity of **16** on the basal activity of the DA-sensitive adenylate cyclase of the rat olfactory tubercle was carried out as described before.[18b] Compound **16** was found to be essentially inactive in this assay, exhibiting 1% stimulation at 100 μM. In the same assay dopamine caused a 50% stimulation of adenylate cyclase at a concentration of 12.5 μM. This finding indicated that compound **16** has little or no in vitro activity on D-1 DA receptors.

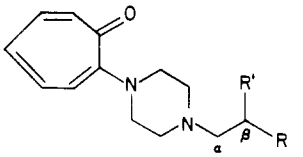
The affinity of compounds **5**, **15**, **16** for rat brain α_1-adrenergic receptors was determined by assessing their ability to displace [^{3}H]WB-4101.[13] These compounds gave IC_{50} values (mean ± SE of three separate experiments) of 1.07 ± 0.09, 1.44 ± 0.01, and 1.09 ± 0.09 μM, respectively. In comparison, the reference α_1-adrenergic receptor antagonist prazosin had an IC_{50} value of 0.65 ± 0.06 mM, (−)-norepinephrine an IC_{50} value of 1.50 ± 0.03 μM, and dopamine an IC_{50} value of 90.0 ± 1.8 μM.

Structure–Activity Relationship. The previously described alkyl-substituted troponylpiperazines[1] did not possess a phenethylamine moiety in their molecule and were considerably less potent than bromocriptine. In contrast, aralkyl-substituted troponylpiperazines, described in the present paper, possess a substituted phenylethylamine moiety. This structural change led to compounds whose potency and efficacy exceeded those of bromocriptine.

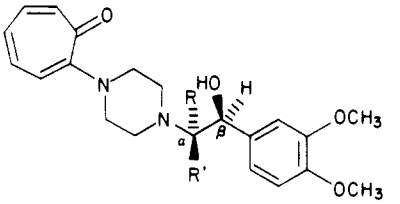
The introduction of the phenylethyl substituent to the troponylpiperazine moiety led to compound **2**, which was inactive (Table I). The introduction of a hydroxyl function α to the nitrogen (**3**) still resulted in an inactive compound. The inactivity of **2** and **3** was surprising since replacement of the phenyl group by a methyl group (as in **31** and **32**) led to compounds that induced intense ambulation in hypokinetic rats[1] (Scheme VI).

Replacement of the phenyl group with a 3,4-dimethoxyphenyl function led to compound **4**, which showed moderate activity. Introduction of a hydroxyl group α to the nitrogen atom (**5**) enhanced the activity. The results generated with compounds **4** and **5** attest to the crucial and accessory roles that the 3,4-dioxa and side-chain hydroxyl groups play, respectively, in eliciting DA agonist activity. The fact that the 3,4-dioxa groups are of paramount importance is supported by the observation that raising the oxidation level of the phenyl ring (**9**) or lowering it (**7** and **8**) leads to substantial decrease or loss of activity.

Scheme VI



31, R = CH_3; R′ = H
2, R = Ph; R′ = H
32, R = CH_3; R′ = OH
3, R = Ph; R′ = OH



13, R = CH_3; R′ = H (threo)
14, R = H; R′ = CH_3 (erythro)
17, R = H; R′ = CH_3 ((+)erythro)
18, R = H; R′ = CH_3 ((−)erythro)

The moderate activity exerted by the 4-methoxyphenyl derivative **7** is of interest. The compound may be considered as a derivative of p-tyramine. As such, it might be a substrate for the enzymes responsible for the biosynthesis of epinephrine. A possible explanation for its activity may be the metabolic transformation of **7** to the dimethoxy derivative **5**—a potent dopaminergic agent in hypokinetic rats.

The dihydroxy derivative **6** was a somewhat weaker DA agonist than the corresponding methylated analogue **4**. This was interesting in view of the fact that, in the dopamine,[14] ADTN,[15] and apomorphine[16] series, methylation of a phenolic hydroxyl leads to a decrease in biological activity and a lack of oral activity. The methoxy derivatives of the present series are orally well absorbed.[17] However, in the DA receptor binding assay compounds **4** and **6** showed comparable activity (see Table V), suggesting either that compound **6** is rapidly metabolized in vivo to an inactive metabolite or that the compound passes the blood–brain barrier only poorly.

It was noted in the alkyl-substituted series[1] that incorporation of a methyl group α to the nitrogen significantly increased DA agonist activity in hypokinetic rats. Incorporation of a methyl group α to the nitrogen atom in compound **5** led to the formation of stereoisomers **13** and **14** of which **14** appeared to be more potent than the desmethyl analogue **5**.

The stereo- and enantiospecificity of the DA receptor site has been previously documented for the DA antagonist butaclamol.[18] The present in vivo results indicate that the receptor site exhibits stereo- and enantiospecificity in the case of DA agonists as well. The threo derivative **13** was much less potent than the erythro isomer **14** (Scheme VI). Furthermore, resolution of racemates **5** and **14** led to the isolation of enantiomers **15**, **16** and **17**, **18**, respectively, of which only enantiomers **16** and **18** exerted potent DA agonist activity. This stereo- as well as enantiospecificity is further reflected in the in vitro DA binding studies with these compounds. Erythro isomer **14** was found to have about 8.0 times greater binding affinity than threo isomer **13**. Similarly, the S-(−) enantiomer **16** had a greater binding affinity compared to the corresponding R-(+) enantiomer **15** by a factor of about 8. It is of interest to

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note that the enantiochemical restraint at the α_1-adrenergic receptors is not so pronounced. Thus, both the enantiomers bind with qualitatively equal affinity to this site. This has also been observed[23] in the (7*R*)-7*H*-indolo[3,4-*gh*][1,4]benzoxazines series. It is suggested from the study of the stimulation of DA-sensitive adenylate cyclase that the potent DA-agonist **16** interact largely with D-2 subpopulation of the DA receptors as it was inactive in stimulating adenylate cyclase.

Replacing the tropone moiety of compound **5** with a phenyl ring as in **19** led to an inactive compound (Table II). Furthermore, changing the piperazine to homopiperazine (**21**), ethylenediamine (**20**), or amino grouping (**22**) also abolished DA agonist activity. The latter results support the previous finding that both the piperazine and the tropone moieties are necessary for dopaminergic activity.[1]

Compound **16** (AY-27, 110) which was recently given the USAN name ciladopa, is being investigated in the clinic as an antiparkinson agent. The pharmacology of ciladopa has recently been described.[17]

Experimental Section

The infrared and ultraviolet spectra were recorded on Perkin-Elmer diffraction grating or Zeiss DMR-21 spectrophotometer, respectively. The melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The NMR and the mass spectra were performed on the CFT-20 and LKB-9000s spectrometer, respectively. Organic extracts were dried over magnesium sulfate and the solvents were always removed under vacuum. Merck silica gel 60 (70–230 mesh) was used for column chromatography. Optical rotations were determined at 1% concentration in DMF at 25 °C with a Perkin-Elmer 241 MC polarimeter.

The general procedure for alkylation of troponylpiperazine was similar to that derived earlier.[1] A typical procedure is described below.

2-[4-(2-Hydroxy-2-phenylethyl)-1-piperazinyl]-2,4,6-cycloheptatrien-1-one (3). A suspension of troponylpiperazine mesylate **1**[1] (11.4 g, 1 eq) in acetonitrile (60 mL) containing potassium carbonate (3.03 g, 4.4 equiv) was heated to 80 °C for 30 min. A solution of α-bromoacetophenone (7.96 g, 1 equiv) in acetonitrile (50 mL) was added dropwise over 30 min. The mixture was stirred at 80 °C for 25 h. The solvent was removed under vacuum. The residue was partitioned between chloroform–water, organic liquor was dried, and the solvent was removed. The residue (11.7 g) was dissolved in ethyl acetate and filtered through a silica gel (400 g) column to yield the ketone (5.8 g, 47.5%): mp 88–92 °C; IR ($CHCl_3$) 1700, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (9 H, m, arom), 3.75 (2 H, s, COCH), 3.35 (4 H, m, CHN), 2.75 (4 H, m, CHN).

To a solution of the above ketone (5.26 g, 1 equiv) in methanol (50 mL) was added sodium borohydride (0.46 g, ~3 equiv) in small portions. The mixture was allowed to stir for a half hour. The solvent was evaporated. The residue was taken up in chloroform–water, the organic layer was washed with saturated ammonium chloride and dried, and the solvent was removed to yield crude product (4.8 g). Two crystallization from methanol gave pure product (2.2 g, 41%): mp 129–131 °C; IR ($CHCl_3$), 3590, 3430, 1565 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.0 (10 H, m, arom), 4.75 (1 H, m, CHO), 3.8 (1 H, br, OH), 3.35 (4 H, m, NCH), 2.6 (6 H, m, NCH). Anal. ($C_{19}H_{22}N_2O_2$) C, H, N.

2-[4-[2-Hydroxy-1-methyl-2-(3,4-dimethoxyphenyl)-ethyl]-1-piperazinyl]-2,4,6-cycloheptatrien-1-one (*threo*-13, *erythro*-14). **A.** To a suspension of bromo ketone **23** (0.819 g, 1 equiv)[3] in acetonitrile (10 mL) was added potassium carbonate (0.912 g, 4.4 equiv) and the mixture was heated to 80 °C. To the hot mixture was added portionwize troponylpiperazine mesylate[1] **1** (0.85 g, 1 equiv) in six portions, every 20 min. The heating was continued for 40 min after the last addition. When no starting material was left as detected by TLC, the heating was stopped and the solvent was removed. The residue was dissolved in chloroform, the solution was washed with water and dried, and the solvent was removed to yield crude product (1.25 g, 52%). A sample was purified by passing through silica gel column and the product was eluted with ethyl acetate to yield an oil: IR ($CHCl_3$) 1652, 1495, 1445, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (8 H, m, arom), 3.9 (6 H, s, OCH_3), 3.35 (4 H, m, NCH), 2.75 (5 H, m, NCH), 1.3 (3 H, d, CCH_3).

The above ketone (16.1 g, 1 equiv) was dissolved in methanol (70 mL). To the solution, sodium borohydride (1.7 g) was gradually added. When no more starting ketone was detected by TLC, the reaction was quenched with saturated ammonium chloride and the solvent was removed. The residue was extracted with chloroform, the extract was washed with water and dried, and the solvent was removed. The residue (13.7 g) was chromatographed on silica gel (700 g) in 50% ethyl acetate–hexane and less polar product (4.5 g, 30%) was eluted with ethyl acetate. It was crystallized from methanol–ether to yield a pure sample: mp 133–135 °C; IR (Nujol) 3340, 1560, 1260, 1150 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 6.85 (8 H, m, arom), 5.0 (1 H, br, OH), 4.23 (1 H, d, HCO, J = 7.0 Hz), 3.83, 3.9 (6 H, 2 × s, OCH_3), 3.4 (4 H, t, NCH), 2.75 (5 H, m, NCH), 0.8 (3 H, d, CCH_3). This product was assigned structure **13** (threo) on the basis of its NMR. Further elution with 10–20% methanol–ethyl acetate gave a more polar product (2.0 g): mp 110–114 °C; IR (Nujol) 3420, 2700, 1578, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.4 (8 H, m, arom), 4.85 (1 H, d, HCO, J = 4.2 Hz), 3.88 (6 H, s, OCH_3), 3.35 (4 H, t, NCH), 2.75 (5 H, m, NCH), 0.9 (3 H, d, CCH_3). The product was assigned structure **14** (erythro).

B. To a suspension of alcohol **28** (4.2 g, 1 equiv) in methanol (20 mL) was added a solution of 2-methoxytropone (2.04 g, 1 equiv) in methanol (5 mL). To the stirred mixture was added a solution of sodium (0.34 g) in methanol (14.6 mL). After refluxing for 3 h, when all the methoxytropone had reacted, the reaction was stopped and the solvent was removed. The residue was crystallized from ethyl acetate–ether to yield the desired product (4.6 g). This sample was identical in all respects with the minor (erythro) alcohol **14** obtained from borohydride reduction.

The alcohol **14** (5.2 g, 1 equiv) was dissolved in acetone (8 mL), and maleic acid (1.73 g, 1.1 equiv) in acetone (9 mL) was added to it. The mixture was stirred for 20 min and ether (150 mL) gradually added to yield crude salt (5 g). Three crystallizations from methanol–ether (5:1) yielded pure product (2.1 g): mp 162–164 °C; IR (Nujol) 3420, 2700, 1578, 1550, 1265 cm^{-1}; UV (MeOH) λ_{max} 343 (9800), 251 (15 500) nm; ^1H NMR (Me_2SO-d_6) δ 6.9 (8 H, m, arom), 6.0 (2 H, s, =CH), 5.2 (1 H, s, OCH), 3.75 (6 H, s, OCH_3), 3.5 (9 H, m, NCH_2), 1.05 (3 H, d, CCH_3). Anal. ($C_{22}H_{28}N_2O_4 \cdot C_4H_4O_4$) C, H, N.

C. Potassium carbonate (0.55 g, 4.4 equiv) was added to a solution of bromohydrin **29** (0.5 g, 1 equiv) in acetonitrile (3 mL). This was followed by the addition of troponylpiperazine mesylate **1** (0.57 g, 1 equiv) and water (1 mL). The reaction was stirred at room temperature for 4 days and finally heated to 80 °C for 6 h. The solvent was removed, diluted with water, and extracted with chloroform. The organic extract dried, and the solvent was removed to give the crude product (0.61 g). TLC of the crude product revealed the presence of only the threo isomer (**13**). A purified sample was spectroscopically (IR, NMR) identical with compound **13**. Anal. ($C_{22}H_{28}N_2O_4$) N.

2-(4-Benzyl-1-piperazinyl)-3′,4′-dimethoxypropiophenone (25). To a solution of bromo ketone **23** (0.546 g, 1 equiv)[3] in acetonitrile (2 mL) was added potassium carbonate (0.304 g, 2.2 equiv). To this mixture was added *N*-benzylpiperazine (0.387 g, 1.1 equiv) and water (0.8 mL). The mixture was stirred at room temperature for 3 h. The solvent was evaporated, and the residue was diluted with water and extracted with chloroform. The chloroform extract was dried and the solvent was removed to yield crude ketone **25** (0.8 g, 100%): ^1H NMR ($CDCl_3$) δ 7.25 (8 H, m,

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arom), 4.0 (1 H, m, NCH), 3.9 (6 H, s, OCH_3), 3.45 (2 H, s, NCH), 2.5 (8 H, m, NCH). This product was used directly for the next step.

2-(1-Piperazinyl)-3′,4′-dimethoxypropiophenone (26). To a solution of ketone **25** (10.6 g) in methanol (90 mL) was added palladium on charcoal (10%, 2.89 g) under a nitrogen atmosphere. To the mixture methanolic hydrochloride acid (2 N, 22 mL) was added, and the mixture was hydrogenated at atmosphere pressure. The reaction was stopped after 5 h, the catalyst was filtered, and the solvent was evaporated. The residue was taken in water and basified with 2 N sodium hydroxide and the product was extracted with chloroform. The organic extract was dried and the solvent was removed to give crude **26** (7.2 g, 94%) as an oil: IR ($CHCl_3$) 1660, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (3 H, m, arom), 3.94 (6 H, s, OCH_3), 2.85 (5 H, m, NCH), 2.55 (4 H, m, NCH), 1.25 (3 H, d, CCH_3).

1-[2-Hydroxy-1-methyl-2-(3,4-dimethoxyphenyl)ethyl]-piperazine (*erythro*-28, *threo*-27). A. The ketone **26** (31.6 g) in acetic acid (210 mL) was hydrogenated in the presence of platinum oxide (6 g) for 48 h. The mixture was filtered, and the catalyst was washed with boiling water. Most of the solvent was removed under vacuum. The residue was dissolved in small amounts of water and basified with 10% sodium hydroxide. The organic matter was extracted with chloroform and dried, and the solvent was evaporated. The residue solidified and was filtered with ether to give alcohol **28** (21.07 g, 66%): mp 135–139 °C; ^1H NMR ($CDCl_3$) δ 6.8 (3 H, m, arom), 4.77 (1 H, d, J = 4 Hz), 3.84, 3.85 (6 H, 2 s, OCH_3). A sample of ketone **26** when reduced with sodium borohydride gave alcohol **27** whose carbinolic proton was located at δ 4.17 (J = 9.7 Hz), suggesting threo configuration.

B. To a solution of ketone **25** (6.09 g) in acetic acid (48 mL) was added platinum oxide (1.45 g). The mixture was hydrogenated for 5 days. The reaction mixture was filtered, and the catalyst was thoroughly washed with hot water. The solvent was removed, and the residue was dissolved in water (20 mL) and basified with sodium hydroxide (5 N, 24 mL). The product was extracted with chloroform and dried, and the solvent was removed to yield alcohol **28**, mp 135–140 °C (2.2 g, 48%).

Resolution of Compound 14. A solution of racemic alcohol **14** (3.8 g) in methanol (4 mL) prepared by heating and was mixed with a solution of ditoluoyl-*l*-tartaric acid (4.5 g) in methanol (4 mL) and ether (9 mL). The mixture was stirred for 20 min. The resulting salt was fully precipitated by adding ether (15 mL) and was filtered to yield a solid (5 g). This was crystallized from methanol (100 mL) to give pure tartrate salt (3 g, mp 195–198 °C). The salt was suspended in water (25 mL) and basified with sodium hydroxide (5 N, 5 mL). The resulting base was extracted with chloroform, dried, and evaporated to yield the free base (1.57 g). A crystallization from ethyl acetate–ether gave pure levo enantiomer **18**: 1.38 g; mp 95–98 °C; $[\alpha]_D$ −82°.

The residue obtained from the filtrates above (from the purification of the crude tartrate salt) was basified to yield the free base (2.67 g). This was treated as described above with ditoluoyl-*d*-tartaric acid (2.69 g). The usual workup yielded the pure tartrate salt (2.8 g, mp 197–198 °C). The basification of the salt gave the dextro enantiomer **17**: 1.3 g; mp 96–98 °C; $[\alpha]_D$ +83°.

In a manner similar to that described for racemate **14**, the above enantiomers were transformed to their maleate salts. Maleate salt **18**: mp 160–162 °C; $[\alpha]_D$ −36.5°. Maleate salt **17**: mp 161–163 °C; $[\alpha]_D$ +37.4°. Anal. ($C_{28}H_{22}N_2O_4$) C, H, N.

Resolution of Compound 5. To a stirred solution of alcohol **5** (3.7 g) in methanol (25 mL) was added a solution of ditoluoyl-*l*-tartaric acid (4.24 g) in methanol (20 mL). The mixture was stirred overnight at room temperature. The resulting salt was precipitated with ether (20 mL) and filtered to give a solid (7.3 g). The crude salt was suspended in methanol (150 mL) and brought to reflux for 5 min and filtered. The solid (3.5 g) thus obtained had a melting point of 195–197 °C and $[\alpha]_D$ +45.8°. This solid was suspended in water (10 mL) and made basic with sodium hydroxide (5 N, 4 mL). The free base (1.67 g) was isolated by extraction with ethyl acetate. Recrystallization from chloroform–hexane yielded dextro enantiomer **15**: 1.1 g; mp 123–125 °C; $[\alpha]_D$ +11.7. Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

The residue obtained from the methanolic filtrate of the purification of crude salt above was basified to yield a solid (1.6 g). This was dissolved in methanol (7 mL) and mixed with a solution of ditoluoyl-*d*-tartaric acid (1.67 g) in methanol (6 mL). The mixture was stirred for 15 min, diluted with ether (20 mL), and filtered to give a solid (2.92 g): mp 193–195 °C; $[\alpha]_D$ −45.0°. Basification of this solid, followed by extraction with ethyl acetate, yielded the free base (1.37 g). A crystallization from chloroform–hexane gave the pure levo enantiomer **16**: 0.95 g; mp 122–124 °C; $[\alpha]_D$ −12.0°. Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

Hydrochloric Salts of Compounds 5, 15, and 16. The free base (3.4 g) was dissolved in methanol (6 mL, 2 N, methanolic HCl) containing hydrochloric acid. The mixture was stirred for 5 min. Ether was gradually added, and the precipitated oil was allowed to crystallize and filtered. The crude salt (3.96 g) thus obtained was crystallized from methanol–ether to give a pure sample (3.6 g). The melting point for racemate **5** was 175–178 °C. The melting point for the salt of **15**, *R*-(+) enantiomer, was 202–204 °C ($[\alpha]_D$ −10.7°) and that of **16** was 194–200 °C ($[\alpha]_D$ +10.7°).

1-(3,4-Dimethoxyphenyl)-2-bromopropan-1-ol (29). To a solution of bromo ketone[3] **23** (1.37 g, 1 equiv) in methanol–chloroform (4:1, 12.5 mL) was added portionwise sodium borohydride (0.096 g); when the reaction was complete (as monitored by TLC in 20% ethyl acetate–hexane), the mixture was neutralized with hydrochloric acid (10%, 8 drops). The solvent was removed, and the residue was diluted with water and extracted with chloroform. The organic layer rewashed with water and dried, and the solvent was evaporated to give a solid residue (**29**, 1.4 g): ^1H NMR ($CDCl_3$) δ 6.8 (3 H, m, arom), 4.45 (1 H, d, HCO), 4.3 (1 H, m, HCBr), 3.82, 3.83 (6 H, 3 s, OCH_3), 1.53 (3 H, d, CCH_3); MS, m/e 274, 276 $(M)^+$, 194 $(M - HBr)^+$, 167 $(M - C_2H_4Br)^+$.

1-(3,4-Dimethoxyphenyl)-1,2-epoxypropane (30). To a solution of sodium (0.092 g, 1 equiv) in methanol (38 mL) was added bromohydrin **29** (1.1 g, 1 equiv). The mixture was stirred at 60 °C for 2.5 h. The solvent was removed, the residue was partitioned between water and ethyl acetate and dried, and the solvent was evaporated to yield an oily residue. This was purified on a silica gel column (20 g) using ethyl acetate–hexane (1:4). The pure product eluted was an oil (**30**, 0.42 g): ^1H NMR ($CDCl_3$) δ 6.75 (3 H, m, arom), 3.9 (1 H, d, HCO), 3.82 (6 H, s, 2 OCH_3), 3.25 (1 H, m, HCO), 1.06 (3 H, d, CCH_3); MS, m/e 194 $(M)^+$, 179 $(M - CH_3)^+$.

2-[[β-(3,4-Dimethoxyphenyl)ethyl]amino]-2,4,6-cycloheptatrien-1-one (22). A solution of 2-(3,4-dimethoxyphenyl)ethylamine (8 g, 1 equiv) and 2-methoxytropone (5.46 g, 0.9 equiv) in benzene (200 mL) was refluxed overnight. The solvent was removed, and the residue crystallized from ethyl acetate–hexane to yield pure product (9.1 g, 98%): mp 110–112 °C; IR ($CHCl_3$) 3300, 1543, 1257, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.69 (8 H, m, arom), 3.85 (6 H, s, OCH_3), 3.5 (2 H, m, NCH), 2.95 (2 H, t, benzylic). Anal. ($C_{17}H_{19}NO_3$) C, H, N.

1-(3,4-Dimethoxyphenyl)-2-(4-phenyl-1-piperazinyl)-ethanol (19). To a solution of α-bromo-3,4-dimethoxyacetophenone (4.77 g, 1 equiv) in acetonitrile (30 mL) was added phenylpiperazine (4.29 g, 1.1 equiv) followed by potassium carbonate (4.59 g, 2.2 equiv). To the mixture was added water (12 mL) and the mixture was stirred for 2 h at room temperature. The solvent was removed under vacuum. The residue was taken up in chloroform and the extract was washed with water and dried, and the solvent was removed. The crude product (7 g, mp 125–132 °C) thus obtained was used directly for the next step: ^1H NMR ($CDCl_3$) δ 7.2 (8 H, m, arom), 3.93 (6 H, s, OCH_3), 3.8 (2 H, s, COCH), 3.25 (4 H, t, NCH), 2.75 (4 H, t, NCH).

To a solution of the ketone (4.4 g) obtained above, in methanol (40 mL), and chloroform (10 mL) was added gradually over a period of 45 min sodium borohydride (0.67 g). The resulting mixture was filtered. The collected solid was dissolved in chloroform, the solution was washed with water and dried, and the solvent was removed to yield the desired product. Crystallization from methanol gave pure product (3 g, 68%): mp 145–157 °C; IR ($CHCl_3$) 3660, 3420, 1265, 1444, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.0 (8 H, m, arom), 4.75 (1 H, t, OCH), 3.87 (6 H, s, OCH_3), 3.25 (4 H, t, NCH), 2.7 (6 H, m, NCH). Anal. ($C_{20}H_{26}N_2O_3$) C, H, N.

Pharmacology Methods. Animals. Experiments were performed on Sprague–Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) housed in air-conditioned quarters. The room was lighted between 0700 and 1900 h daily

and was maintained at a temperature of 24 ± 2 °C.

Materials. In addition to the test compounds, the following drugs were used: 6-hydroxydopamine hydrobromide (Aldrich Chemical Co., Inc.), α-methyltyrosine (Aldrich Chemical Co., Inc.), and bromocriptine methanesulfonate (gift of Sandoz Pharmaceuticals). The doses used were calculated as the free base. The compounds were dissolved in distilled water or suspended in distilled water with a few drops of Tween 80 (2–3 drops/10 mL). Fresh solutions were prepared on the day of the experiment.

6-OHDA-Induced Hypokinesia in Rats. Details of the lesioning procedure and behavioral testing were recently described.[19] Briefly, male rats (ca. 280 g) were anesthetized with sodium pentobarbital and placed in a Stoelting stereotaxic instrument. 6-OHDA (26 μg/4 μL) was injected bilaterally into the anterolateral hypothalamus by using the DeGroot[20] brain atlas. Four days postoperatively, the rats were placed into an open field, the floor of which was divided into 36 squares (11.5 cm × 11.5 cm). The rats were observed visually for a 2-min period and only rats with almost total akinesia were used. Drug effect was evaluated in the course of six 2-min test periods, 15, 30, 45, 60, 90, and 120 min after the sc administration of the test compounds and 2, 3, 4, 5, 6, and 7 h after the sc administration of bromocriptine. The placement of all four limbs in one squares was taken as one ambulation score. The results are expressed as cumulative ambulation scores averaged per rat, which are the sums of the scores obtained during the 2-min observation periods. The compounds responding with less than 100 cumulative ambulation were considered inactive.

Rotational Behavior in Unilaterally 6-OHDA-Lesioned Rats. The body weights of the male rats were approximately 250 g at the time of the stereotaxic operation. During the course of the subsequent rotational experiments, the rats were housed individually and received about 20 g of food per day, which maintained their body weight between 350 and 400 g.

The lesioning procedure was based upon the method of Ungerstedt[8] utilizing the modifications of Pycock and Marsden.[21] The rats were anesthetized with sodium pentobarbital, 40 mg/kg ip, and immobilized in a Stoelting stereotaxic instrument. Unilateral injections of 6-OHDA hydrobromide (8 μg in 3 μL delivered at a rate of 1 μL/min) were made into the left ascending median forebrain bundle (MFB) in the lateral hypothalamus by using the stereotaxic coordinates of the DeGroot[20] brain atlas (A, +4.6; L, 1.9; V_1 −2.7). 6-OHDA was made up in distilled water containing 0.2 μg/μL ascorbic acid and kept in ice through the injection procedure.

Three to four weeks after lesioning, the rats were tested for rotational behavior in response to apomorphine, 0.25 mg/kg sc. Rats that turned 8–10 times per minute during peak activity were selected for further drug trials.

Rotational behavior was determined in automatically recording rotometers, details of which were recently described.[22] Groups of four to eight rats were injected sc with the test compounds and then placed immediately into the rotometer. Rotational behavior was continuously recorded until its cessation. The results are expressed as total number of contralateral turns and rotations per minute during peak activity.

α-Methyltyrosine-Induced Hyperprolactinaemia. Female Sprague–Dawley rats (ca. 200 g) were singly housed with food and water available ad libitum. All experiments were performed in the morning to ensure uniformity between them. The rats were given α-methyltyrosine (200 mg/kg ip) 1 h before the test compounds. At this dose, the prolactin level would be elevated from a basal level of 20 to about 450 ng/mL. The rats were decapitated 30 min after the intraperitoneal or 60 min after the oral administration of the compounds. Trunk blood was collected and was allowed to clot at 4 °C. After centrifugation, serum was obtained and kept frozen until assayed.

Serum prolactin was assayed by a double-antibody radioimmunoassay with materials obtained as gifts from Dr. A. F. Parlow and the National Institutes of Health, Bethesda, MD. The intra- and interassay variabilities were 5% and 12%, respectively. The results are expressed as ED_{50} values, defined as being the dose that lowered the serum prolactin level to 50% of that of the α-methyl-*p*-tyrosine-treated control animals. Each ED_{50} value was determined from one dose–response curve consisting of at least seven different dose points. At least seven to nine rats were used to determine each dose point.

Biochemistry Methods. Materials. In addition to test compounds, the following drugs were used: [^{3}H]haloperidol, specific activity 13.5 Ci/mmol, and [^{3}H]WB-4101, 2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxan, specific activity 25.4 Ci/mmol, were purchased from New England Nuclear. Prazosin was a gift from Pfizer Inc. (−)-Norepinephrine and dopamine were purchased from Sigma Chemical Co.

Method. Sprague–Dawley rat brain membranes and assay procedures for the dopamine and adrenergic receptors binding were carried out as previously described.[12] Rat striatal tissues were used for [^{3}H]haloperidol (DA-receptor binding) and rat whole brain minus cerebellum and brain stem were used for [^{3}H]WB-4101 binding (α_1-adrenergic receptor binding). The final tissue concentration was approximately 20 mg/mL. The incubation time was 10 min for [^{3}H]haloperidol and 15 min for [^{3}H]WB-4101 experiments. Specific binding of [^{3}H]haloperidol (2 nM) was defined with (+)-butaclamol (1.0 μM) and [^{3}H]WB-4101 (0.22 nM) with (−)-norepinephrine (2 μM). The concentration of the compound that produced 50% displacement of the specific binding (IC_{50} value) was determined from the Hill linearization method. Adenylate cyclase activity in homogenate of olfactory tubercle was determined by using method and material previously described in detail.[18b]

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