

Table VIII. Correlation Matrix

	π	σ	MR	IA	$\log 1/I_{50}$
π	1.00				
σ	-0.16	1.00			
MR	0.48	0.16	1.00		
IA	-0.32	-0.08	-0.04	1.00	
$\log 1/I_{50}$	0.50	0.00	0.51	0.57	1.00

The experimental values for NO₂ and Cl, namely -0.28 and 0.71, obtained by subtracting $\log P$ of **1e** from that of **1c** and $\log P$ of **10b** from that of **10d**, respectively, were found to be in agreement with the corresponding values in the literature.¹⁴ The values for

the other substituents were taken from the literature.¹⁴ Different equations were generated by using standard multiparameter regression analysis program on Wang/IBM PC XT computers.

Acknowledgment. One of us (M.S.) is thankful to the Director, CDRI for the award of a research fellowship.

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Chemistry and Pharmacology of the Non-Benzodiazepine Anxiolytic Enciprazine and Related Compounds

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In the course of studies on tranquilizers, new non-benzodiazepine-like compounds were synthesized. These are 1-(3,4,5-trimethoxyphenoxy)-3-[4-(2-methoxyphenyl)piperazinyl]propan-2-ol (INN: enciprazine) and derivatives thereof which were screened pharmacologically in order to evaluate their central nervous system activity. Compounds with marked antiaggressive and anxiolytic properties but without dependence potential could be detected. Enciprazine was selected for clinical investigations.

Introduction

During recent years the synthesis and biological evaluation of 1-(alkoxyphenoxy)-3-(*N*-aryl piperazinyl)propan-2-ols and related compounds have been under investigation in our laboratories.¹ In the course of our studies on tranquilizers we tried to synthesize new non-benzodiazepine-like compounds by combining the β_2 -blocker moiety—characteristic of anxiolytics²—with phenylpiperazines.

A variety of biological activities of (phenoxyphenyl)piperazinyl)propanol derivatives, e.g. local-anaesthetic, hypotensive, and cardiovascular properties, have been reported.³ We were especially interested in derivatives acting on the central nervous system (CNS), as the tranquilizing activities of this class of compounds appeared to be very promising.^{4a,b}

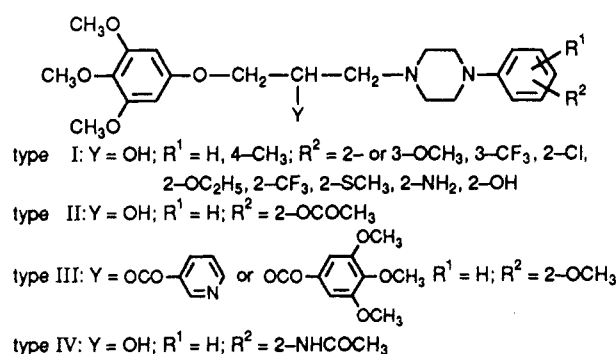
The great interest in the development of non-benzodiazepine-like tranquilizers like buspirone^{4c} led us to study the contribution of substituents in the phenylpiperazine moiety with regard to CNS activities.

This paper deals with the synthesis, the pharmacological screening, and the evaluation of the dependence potential of 1-(3,4,5-trimethoxyphenoxy)-3-(4-phenylpiperazinyl)propan-2-ols of type I, II, III, and IV (Chart I).

Chemistry

According to the synthetic sequence for β -antagonists, condensation of 1-(3,4,5-trimethoxyphenoxy)-2,3-epoxypropane with appropriate phenylpiperazines in 2-propanol led to the synthesis of compounds of type I. The corresponding epoxy compound was prepared by reacting commercially available 3,4,5-trimethoxyphenol (antiarol) with epichlorohydrin in the presence of NaOH. The acylated *o*-hydroxy compounds of type II were obtained by treat-

Chart I



ment of 1-benzyl-4-(2-hydroxyphenyl)piperazine with acid chlorides in pyridine and subsequent condensation of the hydrogenolytically deprotected *N*-phenylpiperazines as described for compounds of type I.

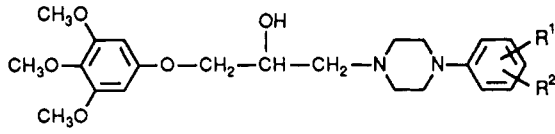
Compounds of type III were synthesized by reaction of 1-(3,4,5-trimethoxyphenoxy)-3-[4-(2-methoxyphenyl)piperazinyl]propan-2-ol with the corresponding (hetero)-aromatic acid chloride in pyridine.

Starting with 1-benzyl-4-(2-nitrophenyl)piperazine, reduction and debenzylation yielded the corresponding aminophenylpiperazine. After condensation according to the

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Table I. 1-(3,4,5-Trimethoxyphenoxy)-3-(4-phenylpiperazinyl)propan-2-ols of Type I


no.	R ¹	R ²	salt	mp, °C	fighting test (mice) ED ₅₀ (mg/kg po)
1	3-OCH ₃	H	2HCl	202–204	5.5 (3.1–7.7)
2	2-OCH ₃	H	2HCl	196–197	1.4 (0.9–1.8)
3	3-CF ₃	H	2HCl	169–172	9.0 (7.3–11.9)
4	3-CH ₃	4-CH ₃	2HCl	185–188	13.0 (9.6–16.7)
5	2-Cl	H	HCl	193–195	16.0 (11.7–20.3)
6	3-CH ₃	H	2HCl	189–192	12.0 (8.3–16.1)
7	2-OC ₂ H ₅	H	2HCl	196–200	19.0 (14.7–24.8)
8	2-CF ₃	H	2HCl	205–206	21.0 (17.3–25.4)
9	2-SCH ₃	H	HCl	183–185	14.0 (9.7–19.3)
10	2-NH ₂	H	HCl	181–183	1.4 (0.69–2.88)
11	2-OH	H		130–131	1.3 (0.58–2.63)

procedure for type I and subsequent N-acylation with acetyl chloride, compounds of type IV were obtained.

All compounds were synthesized and tested as racemates. Investigations regarding optical resolution and enantioselective synthesis will be reported at a later date.

Results and Discussion

Anxiolytic Activity. The purpose of this study was to evaluate the effects of the title compounds on CNS activity by varying the substituents in the (*N*-aryl-piperazinyl)propanol moiety. Tables I and II give the ED₅₀ values (mg/kg po) for the mice fighting test. These results indicate that compounds 2, 10–12 and 15 are as efficient as benzodiazepines in the fighting test (Table III).

Buspirone exhibited weak activity in the fighting test with an oral ED₅₀ value of 14.5 mg/kg. Other investigators found buspirone to be even less active (ED₅₀ = 80.1 mg/kg po).⁵

In the elevated hexagonal maze test, 2.6 mg/kg of diazepam prolonged the time the rats spent on the open arms by 50%. Enciprazine caused a weak prolongation of up to 32% in the dose range between 0.3 and 1.25 mg/kg ip. Oral doses of 1.5–20 mg/kg were ineffective in this model. Buspirone (0.3–2.5 mg/kg ip and 1.5–20 mg/kg po) did not increase the time the rats spent on the open segments.

As demonstrated earlier,⁶ both non-benzodiazepine anxiolytic compounds, enciprazine and buspirone, induced similar changes in the pharmaco-EEG. The mean power values in δ and θ frequency bands decreased and the α and fast β frequency bands increased. The effect of diazepam, however, was characterized by decreases in the α and δ frequency bands and by marked increases in the fast β waves. The pharmaco-EEG changes could be distinguished unambiguously from those of buspirone and enciprazine.

Side Effects

Those compounds which were most efficient with regard to their anxiolytic activity were screened further in the hexobarbital potentiation test, the ethanol potentiation test, and the rotarod test. These tests gave information about sedation and ataxia, which are both undesired side effects of the benzodiazepines diazepam, clobazam, and prazepam. As the data in Table III demonstrate, the most potent anxiolytic compounds showed only weak activity in the above mentioned test models. Therefore they are significantly superior to the benzodiazepines with respect to sedation as undesired side effect.

On the basis of these data, enciprazine (2) was selected for further studies in order to complete its pharmacological profile and to start its clinical investigation.

Dependence Liability

Physical Dependence. Typical dose-dependent withdrawal symptoms such as diarrhea, tremor, jumping, piloerection, restlessness, and salivation were observed in rats treated with diazepam. However, the rats treated with enciprazine showed no withdrawal symptoms after deprivation of the compound previously administered up to a dose of 2 × 50 mg/kg po per day (Table IV). Both compounds, enciprazine and diazepam, caused no weight loss after withdrawal in rats.

Psychic Dependence. Drug self-administration studies in animals are an accepted method for the preclinical evaluation of the psychic dependence potential of a drug.⁷ The validity of the approach is based upon the observation that drugs being self-administered by laboratory animals (i.e., have reinforcing properties) are those commonly abused by humans. Further, drugs which are found to give rise to aversion in humans are avoided by laboratory animals as well.⁸

Doses of enciprazine that were tested ranged from 0.001 to 1.0 mg/kg per injection. They were chosen on the basis of pharmacological results indicating that enciprazine has a similar potency as diazepam. In a previous experiment with diazepam, maximum rates of responding were maintained by 0.01–0.1 mg/kg per injection.⁹

Table V shows that responding was maintained by 0.3 mg/kg of pentobarbital (exception: monkey #9083 with 0.1 mg/kg). The mean number of injections of flurazepam the monkeys self-administered was in general higher than that for saline. For five monkeys the difference was significant, for one monkey (#9083) it was only small (Table VI). All monkeys self-administered enciprazine to the same extent as saline. There was no indication of a dose-response function for enciprazine (Table VII).

These data clearly indicate that enciprazine is missing reinforcing properties under the conditions of the experiment. The monkeys which refused to self-administer enciprazine did respond to flurazepam (exception: monkey #9083. However, in previous studies this monkey did self-administer other anxiolytics such as lorazepam).

Structure-Activity Relationship (SAR)

Our pharmacological results with respect to the anxiolytic activity of 1-(3,4,5-trimethoxyphenoxy)-3-(4-phenylpiperazinyl)propan-2-ols (see Tables I and II) led us to investigate possible structure-activity relationships.

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Table II. 1-(3,4,5-Trimethoxyphenyl)-3-(4-phenylpiperazinyl)propan-2-ols and Their Derivatives of Type II-IV


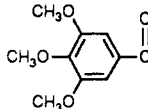
$ \begin{array}{c} \text{CH}_3\text{O} \\ \\ \text{CH}_3\text{O}-\text{C}_6\text{H}_2-\text{O}-\text{CH}_2-\text{CH}(\text{OR}^1)-\text{CH}_2-\text{N}(\text{piperazine})-\text{C}_6\text{H}_4-\text{R}^2 \\ \\ \text{CH}_3\text{O} \end{array} $					
no.	R ¹	R ²	salt	mp, °C	fighting test (mice ED ₅₀) (mg/kg po)
12	H	2-OCOCH ₃	HCl	189–191	3.2 (1.19–5.72)
13		2-OCH ₃	3HCl	187–192 dec	14.0 (9.4–17.88)
14		2-OCH ₃	2HCl	193–195	16.0 (13.27–18.96)
15	H	2-NHCOCH ₃		127–131	3.3 (1.46–5.72)

Table III. Anxiolytic Activity, Side Effects, and the Resulting Therapeutic Indices of Some Test Compounds and Reference Samples

compound	anxiolytic activity		side effects, ED ₅₀ (mg/kg po) ^d			therapeutic indices		
	fighting test ^d ED ₅₀ (mg/kg po)	maze test ^e % time prolong.	hexobarbital potentiation	ethanol potentiation	rotarod	ED ₅₀ (hexob) ED ₅₀ (fight.)	ED ₅₀ (ethanol) ED ₅₀ (fight.)	ED ₅₀ (rot.) ED ₅₀ (fight.)
2	1.4 (0.71–2.77)	32 ^b (0.3 mg/kg ip)	46.4 (36.0–59.6)	45.5 (26.6–77.9)	120 (89.6–160.6)	33	32.5	86
3	9.0 (7.3–11.9)		90 (76.3–106.2)		400 (355.1–460.2)	10		44
10	1.4 (0.69–2.88)		145 (119.4–168.3)		260 (231.4–298.5)	103		185
11	1.3 (0.58–2.63)		200 (172.3–227.4)		100 (86.2–116.6)	154		77
12	3.2 (1.19–5.21)							
15	3.3 (1.46–5.72)		200 (181.4–219.9)		100 (87.7–114.6)	61		30
diazepam	1.4 (0.92–2.07)	50 ^c (2.6 mg/kg po)	3.0 (1.93–4.60)	0.85 (0.41–1.79)	4.0 (2.99–5.35)	2.1	0.6	2.9
clobazam	2.35 (1.03–4.77)		13.2 (9.6–17.1)		4.1 (2.2–6.4)	5.6		1.8
prazepam	8.0 (6.62–10.07)		11.5 (8.3–15.4)		3.1 (1.1–5.3)	1.4		0.4
buspirone	14.5 (80.1 ^a) (10.32–18.72)	no effect	(54.5 ^a)	(62.8 ^a)	77 (64.3–92.1)	(0.7)	(0.8)	5.3

^a See ref 17. ^b *p* < 0.05. ^c *p* < 0.01. ^d *n* = 10 mice/dose. ^e *n* = 6 rats/dose.**Table IV.** Comparison of the Physical Dependence Potential of Enciprazine and Diazepam in Rats

withdrawal symptoms	diazepam ^a		enciprazine ^a	
	10	20	50	100
diarrhea	+	++	–	–
tremor	–	+	–	–
jumping	–	+	–	–
piloerection	+	++	–	–
restlessness	+	+	–	–
salivation	–	+	–	–

^a Daily dose, given in milligrams per kilogram (po), was divided into two equal parts. Legend: –, no effect; +, weak effect (three of six animals showed symptoms); ++, strong effect (more than three of six animals showed symptoms).

We focused on those test compounds bearing a 2-substituted phenyl ring in the phenylpiperazinyl moiety (see Table VIII). These nine propan-2-ols show a broad spectrum of anxiolytic activity (ED₅₀ = 1.3–21.0).

For establishing a structure-activity relationship, physicochemical parameters have to be applied. These may be electronic, steric, or hydrophobic indices.¹⁰

Of the great variety of hydrophobic parameters, a very common one is π , which is defined as $\pi = \log (P/P_0)$.¹⁰ π is correlated with the partition coefficient *P*, being determined in an octanol/water system (*P*₀ refers to the standard, i.e. the unsubstituted phenyl ring). The π values for the phenyl substituents of the compounds in Table VIII are taken from the literature.¹¹

Linear-regression analysis revealed a good correlation between anxiolytic activity [$\log [1/\text{ED}_{50}(\text{Fight.})]$] and π . Figure 1 shows that the anxiolytic activity increases with decreasing hydrophobicity of the *o*-phenyl substituent. Although this simple model neglects other factors like

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Table V. Drug Self-Administration Test in Monkeys: Mean Number of Injections of Pentobarbital and Saline per Session

monkey	pentobarbital ^a		saline ^b
	0.1 mg/kg	0.3 mg/kg	
±2036		44.9 (±5.6)	5 (1-9)
±2037		60.1 (±5.3)	20 (5-23)
±3017		76.6 (±15.9)	8.5 (0-18)
±3038		53.9 (±9.3)	3.6 (0-9)
±9079		56.5 (±18.0)	4 (1-11)
±9083	59.8 (±15.8)		13 (4-19)

^aThe standard deviation is given in parentheses. ^bThe numbers in parentheses indicate the range of variation.

Table VI. Drug Self-Administration Test in Monkeys: Mean Number of Injections^a of Flurazepam and Saline per Session

monkey	flurazepam					saline
	0.01 ^b	0.03 ^b	0.1 ^b	0.3 ^b	0.56 ^b	
±2036	26 (25-28)	33 (30-36)	40 (37-43)	11 (10-12)		5 (1-9)
±2037	40.7 (33-51)	68 (63-73)	33			20 (5-23)
±3017	17.7 (14-22)	27.5 (26-29)	54.7 (40-47)	40.3 (28-50)		8.5 (0-18)
±3038		8.0 (0-14)	50 (42-58)	22 (12-32)		3.6 (0-9)
±9079			12.5 (5-20)	44.7 (32-53)	16.7 (15-18)	4 (1-11)
±9083		29 (19-47)	26.3 (20-32)	18.3 (14-23)		13 (4-19)

^aThe numbers in parentheses indicate the range of variation.

^bMilligrams per kilogram per injection.

steric and electronic influences, it helps us to understand the differences in the activity of the test compounds.

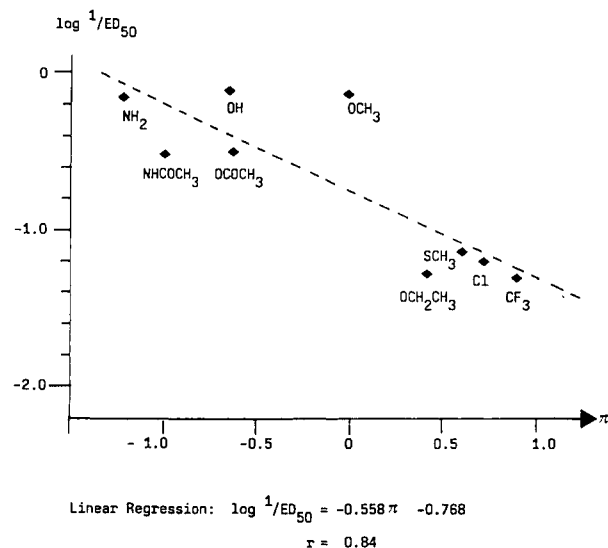
Another method to determine hydrophobicity constants is based on chromatographic retention.^{12,13} We measured R_f values of the test compounds on reversed-phase TLC plates by applying different mobile phases. These data were used to calculate R_{m0} values (Table VIII). The R_{m0} value increases with increasing lipophilicity/hydrophobicity of a compound. Again, linear-regression analysis showed satisfactory correlation ($r = 0.86$) between anxiolytic activity and the lipophilicity parameter R_{m0} .

Thus, both methods, using π values as well as R_{m0} data, are able to reveal a correlation between lipophilicity and biological activity of the listed 1-(alkoxyphenoxy)-3-(4-phenylpiperazinyl)propan-2-ols. This structure-activity relationship may be helpful for future research in this area.

Experimental Section

1. Chemical Section. General Procedures. All melting points were determined with a Dr. Tottoli melting apparatus. They are uncorrected. Microanalyses were obtained by a Perkin-Elmer 240 analyzer. All compounds were obtained as racemates.

1-(3,4,5-Trimethoxyphenoxy)-2,3-epoxypropane. In a reaction vessel suitable for azeotropic separation of water, 18.4 g (0.1 mol) of 3,4,5-trimethoxyphenol is boiled with 37 g (0.4 mol) of epichlorohydrin, followed by dropwise addition of 10 g (0.1 mol) of 40% sodium hydroxide over a period of 30 min. Simultane-

**Figure 1.** Correlation between biological activity (ED_{50} of fighting test) and π .

ously, the water is removed azeotropically. After complete addition, the mixture is left to react for another hour at boiling temperature. Then it is diluted with approximately 100 mL of toluene. NaCl precipitates and is filtered off. The filtrate is fractionated first under normal pressure and then in vacuo. 1-(3,4,5-Trimethoxyphenoxy)-2,3-epoxypropane is obtained as colorless oil at $bp_{1.0} = 175-180^\circ\text{C}$. Yield: 19.2 g (80% based on trimethoxyphenol).

1-(3,4,5-Trimethoxyphenoxy)-3-[4-(3-methoxyphenyl)-piperazinyl]propan-2-ol (1). 1-(3,4,5-Trimethoxyphenoxy)-2,3-epoxypropane (12 g, 0.05 mol) and 1-(3-methoxyphenyl)-piperazine (9.6 g, 0.05 mol) are heated to reflux for 5 h in 100 mL of 2-propanol. Most of the solvent is distilled off; the residue is treated with an excess of 2-propanolic HCl, and the dihydrochloride of 1-(3,4,5-trimethoxyphenoxy)-3-[4-(3-methoxyphenyl)piperazinyl]propan-2-ol is precipitated by addition of diethyl ether, yielding 18.4 g (73%) of a colorless, crystalline substance. Mp: $202-204^\circ\text{C}$. Anal. ($\text{C}_{23}\text{H}_{34}\text{O}_6\text{N}_2\text{Cl}_2$): C, H, N.

Compounds 2-9, 11, and 12 are obtained in the same way as described for 1, starting from 0.05 mol of 1-(3,4,5-trimethoxyphenoxy)-2,3-epoxypropane and 0.05 mol of the corresponding phenylpiperazine.

1-(3,4,5-Trimethoxyphenoxy)-3-[4-(2-aminophenyl)-piperazinyl]propan-2-ol (10). 1-(3,4,5-Trimethoxyphenoxy)-3-[4-(2-nitrophenyl)piperazinyl]propan-2-ol monohydrochloride (6 g, 0.012 mol; prepared according to the method for 1. Mp: 198°C . Yield: 55%) is dissolved in 300 mL of methanol and hydrogenated in the presence of 0.5 g of Pd-carbon (10%) at room temperature and atmospheric pressure.

After removal of the catalyst and evaporation of the solvent in vacuo, the monohydrochloride is isolated from ethanol. Yield: 5.3 g (94%). Mp (monohydrochloride): $181-183^\circ\text{C}$.

1-(3,4,5-Trimethoxyphenoxy)-2-(nicotinoyloxy)-3-[4-(2-methoxyphenyl)piperazinyl]propane (13). 1-(3,4,5-Trimethoxyphenoxy)-3-[4-(2-methoxyphenyl)piperazinyl]propan-2-ol (13.0 g, 0.03 mol, the base of 2) and triethylamine (3.34 g, 0.03 mol) are dissolved in 80 mL of anhydrous benzene, followed by addition of a solution of 4.67 g (0.033 mol) of nicotinic acid chloride in 50 mL of anhydrous benzene over a period of 30 min. After stirring for another 2 h at room temperature, the mixture is heated for 1 h to $70-80^\circ\text{C}$. After cooling to room temperature, the mixture is extracted with water several times and washed with aqueous NaHCO_3 and water; the benzene phase is dried with magnesium sulfate and concentrated. The solid residue is taken up in dioxane. After adding excessive 2-propanolic hydrochloric acid and ether, 13.0 g (67%) of the above-mentioned compound is obtained as the trihydrochloride (colorless crystals). Mp: $187-192^\circ\text{C}$ dec. Anal. ($\text{C}_{29}\text{H}_{38}\text{O}_7\text{N}_3\text{Cl}_3$): C, H, N.

1-(3,4,5-Trimethoxyphenoxy)-2-[(3,4,5-trimethoxybenzoyloxy)-3-[4-(2-methoxyphenyl)piperazinyl]propane (14). 1-(3,4,5-Trimethoxyphenoxy)-3-[4-(2-methoxyphenyl)-

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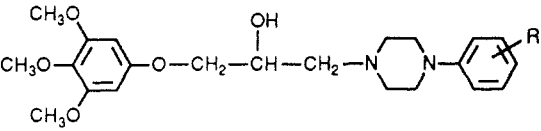
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Table VII. Drug Self-Administration Test in Monkeys: Mean Number of Injections^a of Enciprazine (2) and Saline per Session

monkey	enciprazine (2)							saline
	0.001 ^b	0.003 ^b	0.01 ^b	0.03 ^b	0.1 ^b	0.3 ^b	1.0 ^b	
±2036		7 (5-9)		2.3 (2-3)		3.0 (1-5)		5 (1-9)
±2037		4.3 (2-6)		7.7 (7-9)		2 (0-4)		20 (5-23)
±3017	6.7 (0-17)	1.7 (1-2)		4.3 (1-7)		0.3 (0-1)		8.5 (0-18)
±3038	3.5 (2-5)	4.0 (1-7)		4.3 (1-10)		1.3 (1-2)		3.6 (0-9)
±9079		3.0 (1-6)	2.0 (1-3)	13.7 (2-24)		1.0 (0-2)	1.3 (0-3)	4 (1-11)
±9083		17.7 (13-21)		9 (7-11)		2.7 (1-4)		13 (4-19)

^a The numbers in parentheses indicate the range of variation. ^b Milligrams per kilogram per injection.

Table VIII. Fighting Test Results (ED₅₀) and Lipophilicity Parameters (π , Rm₀) of Some Test Compounds

				
no.	R	π	Rm ₀	fighting test (mice) ED ₅₀ (mg/kg po)
2	2-OCH ₃	1.9563	-0.02	1.4
5	2-Cl	2.671	0.71	16.0
7	2-OCH ₂ CH ₃	2.3845	0.38	19.0
8	2-CF ₃	3.5975	0.88	21.0
9	2-SCH ₃	2.6337	0.61	14.0
10	2-NH ₂	1.592	-1.23	1.4
11	2-OH	1.4391	-0.67	1.3
12	2-OCOCH ₃	2.0115	-0.64	3.2
15	2-NHCOCH ₃	1.743	-0.97	3.3

piperazinyl]propan-2-ol (base of 2) is reacted with 3,4,5-trimethoxybenzoyl chloride in the presence of triethylamine as described for 13. The reaction product is obtained as the dihydrochloride melting at 193-195 °C (dec). Yield: 8.0 g (38%). Anal. (C₃₃H₄₄O₁₀N₂Cl₂): C, H, N.

1-(3,4,5-Trimethoxyphenoxy)-3-[4-(2-acetamidophenyl)-piperazinyl]propan-2-ol (15). Amino derivative 10 (monohydrochloride; 4 g, 0.009 mol) is dissolved in 200 mL of dioxane and treated with 10 mL of triethylamine. Acetylchloride (0.9 mL) is added dropwise at -5 °C while being stirred. After 2 h at room temperature, the solution is filtered, and the solvent is removed under reduced pressure. The product is isolated by column chromatography on silica gel (ether-acetic acid, 1:1). It is recrystallized from acetone-ether. Yield: 2.03 g (50%). Mp: 127-131 °C. Anal. (C₂₄H₃₃N₃O₆): C, H, N.

2. Pharmacological Section. Anxiolytic Activity. The antiaggressive anxiolytic activities of compound 1-15 (see Tables I and II) were determined in the electroshock-induced fighting test¹⁴ in mice. Sixty minutes after oral administration, the male fighting pairs were excited electrically by applying a current for 3 min. The number of pairs showing no fighting activity during the observation period is considered to be a measure for antiaggressive activity. These data were taken to calculate a dose-response curve and the ED₅₀ values were determined by probit analysis.¹⁵ Further evaluation of the anxiolytic activity was achieved in an elevated, six-arm radial maze with female rats. The arms are connected by a hexagonal gangway. Open arms and arms protected by side walls (height: 13 cm) alternate. In the same way "open" and "protected" segments of the surrounding gangway alternate. One hour after oral administration of the test compound the rat is placed in the middle of the elevated hexagonal maze. Exploration behavior of the rat in this unknown environment is

observed for a 3-min test period. Whenever the rat has passed a segment, it has to choose either to enter an open segment without boundary or a closed one. The time spent on the open arms is suggested to be predictive for the anxiolytic activity of the test compound.¹⁶ The time the control rats spent on the open segments was taken as baseline value. Drug-induced prolongation of this time was expressed as percent changes from baseline. The significance of the data was checked by Student's *t* test for nonpaired samples.¹⁷

Side Effects. As described by Gross et al.,¹⁸ trained animals (six mice/dose) were placed on a rotating rod 60 min after oral administration of the test compound and then observed for 2 min. The number of animals in each single dose group that fell from the rod is taken to calculate the ataxic properties of the test compound.

According to the method of Osterloh,¹⁹ a dose of hexobarbital was injected into mice which does not cause lateral position in the control group (35 mg/kg ip). One hour after oral administration of the test compound and 30 min after intraperitoneal application of hexobarbital, the number of sleeping animals (lateral position) in each single dose group was checked.

The experimental procedure in the ethanol-induced sleeping test is based on the method of Barzhagi.²⁰ Mice were treated orally with the test compounds and 30 min later with 4 g/kg of ethanol. The alcohol dose should not induce lateral position in the control animals. One hour after receiving alcohol it was determined how many animals in each group were in a lateral position.

The ED₅₀ values in the three tests were calculated by probit analysis.¹⁵ The therapeutic index is defined as the quotient of the ED₅₀ values of the side effects and the corresponding value of the fighting test.

Dependence Liability Evaluation. Physical Dependence. The test compounds diazepam and enciprazine (2) were orally administered to rats twice a day 6 days a week, and observations were made for 45 days. Two groups of animals (six rats/group) received 2 × 5 and 2 × 10 mg/kg po of diazepam, respectively. Two other groups got 2 × 25 and 2 × 50 mg/kg po of enciprazine, respectively. On the 7th day of each week the test compounds were withdrawn. Typical symptoms of withdrawal such as diarrhea, tremor, jumping, piloerection, restlessness, and salivation were observed and recorded. The significance was verified by Student's *t* test for nonpaired samples.¹⁷

Drug Self-Administration. The most commonly used procedure is a substitution procedure according to Johanson and Balster.⁸ In this procedure, monkeys are trained to self administer

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a drug with known reinforcing properties (termed baseline drug), and then this drug is substituted by the test compound for several sessions in order to determine its ability to maintain self-administration. In this study, the baseline drug was pentobarbital, and flurazepam was used as positive control. Pentobarbital combines reinforcing properties of barbiturate-like and benzodiazepine-like compounds.^{21a,b} The procedure is carried out according to the method of Bergman and Johanson.⁹

When the baseline drug is substituted by the test drug or saline,

it takes several sessions before response rates stabilize. For this reason only the last two or three sessions of each substitution period are used for data analysis.

Data for each animal were analyzed separately. A drug was considered to act as a positive reinforcer only if it maintained responding to a larger extent than that being observed during the last two or three saline control sessions. Six rhesus monkeys trained to self-administer pentobarbital were used to test enciprazine. All monkeys had participated in previous substitution studies involving one to four anxiolytics (e.g., midazolam, halazepam). In all cases, the anxiolytics acted as positive reinforcers. For three monkeys (#2036, 9079, 9083) the dose-response function was determined first for enciprazine and then for flurazepam. The order was reversed for the other three monkeys. Saline was given twice during the study as negative control.

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