2(*R*,*S*)[^{14}C-*methyl*-2-^{14}C],5,5-Trimethylthiazolidine-4-(*S*)-carboxylic Acid ([^{14}C]1). A solution of D-(−)-penicillamine (149.2 mg, 1.00 mmol) dissolved in 2.0 mL of water in a round-bottomed flask adapted with a short liquid-addition tube with drip tip and stoppered at the other end was cooled in an ice bath. The ampule of [1,2-^{14}C]acetaldehyde of specific activity 9.5 mCi/mmol [4.6 mg (0.1 mmol) dissolved in 1.0 mL of water] was frozen in a dry ice/acetone bath. The ampule was then broken, inverted, and placed into the addition tube. The system was stoppered and tilted at a 45° angle to allow the radioactive solution to drip into the reaction flask as it thawed. After 5 min, the tube was rinsed with acetaldehyde stock solution (1.0 mL, 0.0396 g, 9.00 mmol) made by diluting 0.51 mL of freshly distilled acetaldehyde to 10.0 mL with H_2O. Total acetaldehyde = 0.0442 g, 1.00 mmol. After an additional 5 min, the vials and addition tubes were rinsed with cold water (0.5 mL each) and removed. Stirring was maintained in the cold for 1 h and at room temperature for 1.5 h. The reaction mixture was then concentrated to dryness in vacuo and the solid residue recrystallized from ethyl acetate to give 96.7 mg (55.2% yield) of [^{14}C]1: mp 165–166 °C; specific activity 6.00×10^6 cpm/mg (0.47 mCi/mmol). A second crop of lower specific radioactivity was obtained by addition of 100 mg of unlabeled 1 to the residue after evaporation of the mother liquor above, followed by recrystallization (98 mg; 0.26 mCi/mmol). A repeat procedure yielded a third crop (93 mg, 0.14 mCi/mmol). TLC of crops 1 and 2 in *n*-BuOH/HOAc/H_2O (50:11:25) and *n*-PrOH/H_2O (7:3) showed only single radioactive spots, R_f 0.52 and 0.75, respectively, when scanned with a radiochromatogram scanner.

Metabolic Disposition of [^{14}C]1 in the Rat. The metabolism of 1 by the rat was investigated in two separate experiments using ^{14}C-labeled 1. The injection solution was made up of 700.3 mg of unlabeled carrier 1 and 0.70 mg of [^{14}C]1 in water (5 mL). Administration of this solution at 1.0 mL/100 g body weight represented a dose of 8.0 mmol/kg and provided radioactivity of 5.96×10^6 dpm by actual assay (duplicate 500-μL samples of the injection solution were counted).

A male rat of Sprague–Dawley descent weighing 207 g was fasted overnight. After administration of [^{14}C]1, the animal was placed in an all-glass metabolism cage that allowed the separate collection of urine, feces, and expired air. CO_2 was collected in two successive traps containing cold methoxyethanol/ethanolamine (2:1).[28] Sample collections were made at 2, 4, 8, 16, and 24 h after drug administration. At each collection time, the CO_2-trapping solution was removed from both the primary and secondary traps, the volume recorded, and fresh solution added. After the sides of the metabolism cage were rinsed with a small amount of water, urine volume was recorded and the urine stored over Na_2CO_3 (200 mg). The radioactivity of duplicate samples of urine and the CO_2-trapping solutions was determined in Aquasol by liquid scintillation spectrometry. Feces collected over 24 h were lyophilized and pulverized, and 250-mg samples were combusted to $^{14}CO_2$ for radioactivity determinations.

The 24-h urines were pooled, and carrier 1 (400 mg) was added. The solution was thoroughly mixed, filtered to remove debris, adjusted to 100 mL with water, and assayed for total radioactivity. After acetylation, the acetylated 1 was recrystallized to constant specific radioactivity as described previously for the isolation of radioactive 1 from rat urine.[15c] This experiment was repeated with a second rat weighing 247 g, and the results (averaged from both experiments) are presented in Table I.

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Tricyclic Compounds as Selective Antimuscarinics. 1. Structural Requirements for Selectivity toward the Muscarinic Acetylcholine Receptor in a Series of Pirenzepine and Imipramine Analogues

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The M_1-selective antiulcer drug pirenzepine (1) is a tricyclic compound with close resemblance to tricyclic psychotropic agents such as imipramine (2). Despite this fact, pirenzepine is devoid of any psychotropic effects, exhibiting measurable antagonistic effects in biochemical assays and receptor binding studies only toward the muscarinic receptor system. To understand how different groups in these tricyclic molecules affect binding affinities, a set of nine compounds structurally related to pirenzepine (1) and imipramine (2) has been selected for analysis, comprising three different tricycles and three different side chains. The compounds were tested for their affinity to the imipramine and muscarinic receptors in homogenized rat cortex tissue. The result of these studies suggests that it is the nature and placement of accessory groups that determine the differences in receptor recognition and the binding process. In the case of pirenzepine (1), preferential binding toward the muscarinic receptor is brought about by the endocyclic amide group, by the positioning of the protonated N atom of the side chain, and to a minor extent by the exocyclic amide group. From these findings a putative model for the explanation of selective binding of pirenzepine (1) to the muscarinic receptor has been derived.

Pirenzepine (1) (Gastrozepin) is the first M_1-selective muscarinic receptor antagonist that has been introduced into ulcer therapy, providing safe and unproblematic treatment of gastritis and duodenal and peptic ulcer.[1] Pirenzepine selectively inhibits vagally stimulated gastric secretion and various neuronal muscarinic responses but has a lower potency for heart and smooth muscle receptors.[2,3] The selectivity found in whole animal pharma-

† Deceased.

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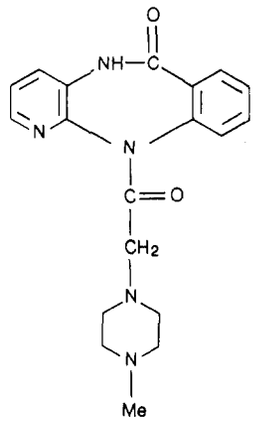
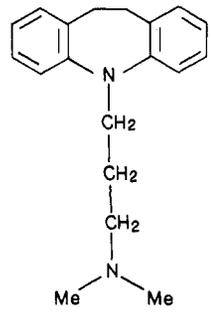


pirenzepine (1)
antimuscarinic
properties only



imipramine (2)
anti - histaminergic,
- dopaminergic,
- adrenergic,
- serotonergic,
- muscarinic properties

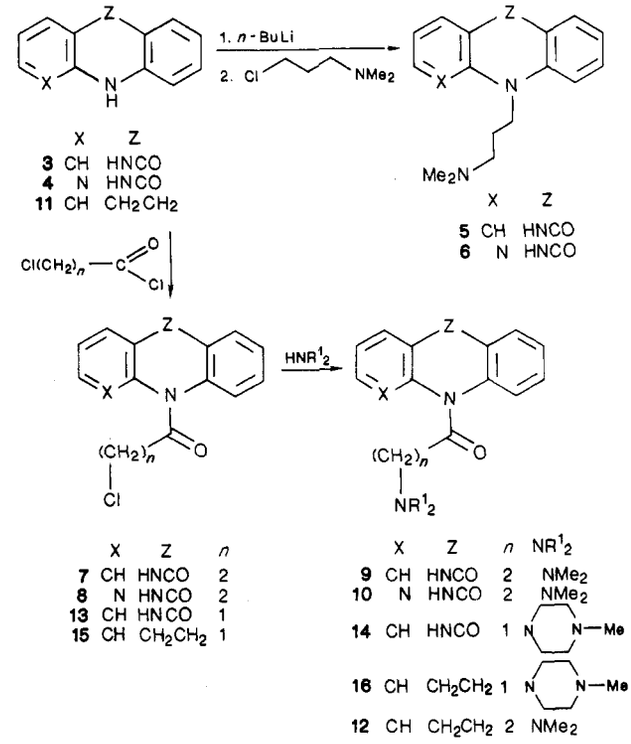
cology could be demonstrated in biochemical assays of receptor function as well as in binding experiments with membrane preparations from different tissues.[4,5]

As indicated in Figure 1, pirenzepine (1) is a tricyclic compound with close resemblance to tricyclic psychotropic agents such as imipramine (2). In general, the majority of these molecules consist of a central six- or seven-membered ring linearly fused to two six-membered rings on either side and a basic nitrogen in a side chain connected to the central ring. In view of this fact, the question arises about possible psychotropic properties of pirenzepine. Under physiological conditions pirenzepine penetrates only very poorly through the blood–brain barrier in various species, including humans.[6] Nevertheless, measurable amounts of the drug could be detected in total brain or in the cerebrospinal fluid in animals and in humans[7] after iv administration. Hence, in therapy a central effect could not a priori be excluded. However, until now no antidepressant nor any other psychotropic effect has been reported following pirenzepine treatment in humans.

The various tricyclic drugs that are used in the treatment of central nervous system (CNS) disorders exhibit an abundant variety of pharmacological properties, which include inhibition of neuronal uptake of norepinephrine and/or serotonin, antagonism of the action of reserpine, and anticholinergic, antidopaminergic, and antihistaminic action as well as cardiotoxic effects.[8]

Recently pirenzepine and some prototype tricyclic psychotropics have been investigated with respect to a large variety of these biochemical parameters. The outcome of this study led to the interesting result that, in contrast to CNS active drugs, pirenzepine exhibits measurable inhibitory effects in biochemical assays and substantial affinities in receptor binding studies exclusively toward the muscarinic receptor system.[9]

Scheme I



First attempts to interpret these findings have recently been undertaken. A spatial analysis performed by semiempirical quantum chemical calculations with the MNDO and PCILO approximations indicated that the energetically favorable conformations of pirenzepine are not found in the class of tricyclic compounds with neuroleptic, antidepressant, or antihistaminic activity.[10]

In order to gain more insight into those structural entities that are decisive for this unique selectivity of pirenzepine, a program has been established for the design, synthesis, and biochemical evaluation of some pirenzepine analogues that seemed to be appropriate for the solution of this problem.

As postsynaptic receptor-mediated effects are difficult to measure, especially in the central nervous system, receptor binding studies have been used for the characterization of the drug receptor interaction. In order to restrict the investigation to a prototype system, the affinity has been measured for the imipramine (IM) and the muscarinic (M) receptors in rat cortex using [^{3}H]imipramine and [^{3}H]-*N*-methylscopolamine, respectively, as radioligands. There is firm evidence that [^{3}H]imipramine binds with high affinity to a site that is associated with the neuronal uptake of serotonin, a process that according to the current view is associated with depression.[11] M cortex receptors are similar to ganglionic M receptors that are thought to be responsible for the modulation of vagally induced acid secretion.[12]

Chemistry

Table I lists the formulas and physical data for the new compounds. The synthesis is outlined in Scheme I. Acylation of tricycles **3**, **4**, and **11** was readily effected by

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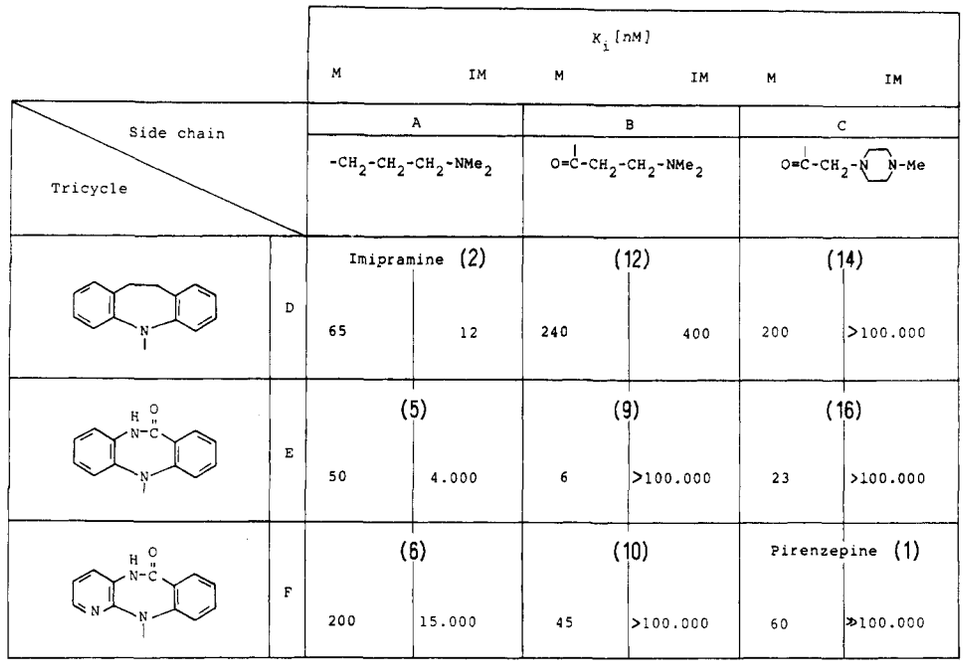
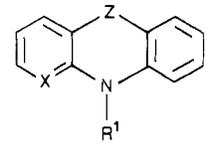


Figure 1. Binding affinities of a set of analogues of imipramine and pirenzepine to the imipramine and muscarinic receptors from homogenized rat cortex tissue. Binding affinities are expressed as K_i values (nM). Standard errors for IC_{50} values were less than 10% of the mean values shown. M = muscarinic receptor, IM = imipramine receptor, $K_i = IC_{50}/(1 + [L]/K_D)$. As can be seen, stepwise exchange of functional groups in imipramine lowers the affinity for the imipramine receptor to a large extent whereas the affinity to the muscarinic receptor is influenced only marginally.

Table I. Derivatives of Pirenzepine and Imipramine



compd	Z	X	R^1	% yield[a]	mp, °C	formula[b]
5	NHCO	CH	$(CH_2)_3N(CH_3)_2$	38	143–146	$C_{18}H_{21}N_3O$
6	NHCO	N	$(CH_2)_3N(CH_3)_2$	23	135–136	$C_{17}H_{20}N_4O$
9	NHCO	CH	$CO(CH_2)_2N(CH_3)_2$	52	256–257	$C_{18}H_{19}N_3O_2 \cdot HCl$
10	NHCO	N	$CO(CH_2)_2N(CH_3)_2$	54	193–194	$C_{17}H_{18}N_4O_2$
12	CH_2CH_2	CH	$CO(CH_2)_2N(CH_3)_2$	89	185–187	$C_{19}H_{22}N_2O$
14	NHCO	CH	$CO(CH_2)_2$-c-$N(CH_2)_2N(CH_3)(CH_2)_2$	32	126–127	$C_{20}H_{22}N_4O_2$
16	CH_2CH_2	CH	$COCH_2$-c-$N(CH_2)_2N(CH_3)(CH_2)_2$	84	98–99	$C_{21}H_{25}N_3O$

[a] No attempt was made to optimize yields. Numbers represent the yield of the last step. [b] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values.

reaction with the corresponding chloro- or bromoacyl halides leading to the intermediate acyl halides **7**, **8**, **13**, and **15**, which in turn by reaction with secondary amines furnished the expected basic tricycles **9**, **10**, **12**, **14**, and **16**. Regioselective alkylation of tricycles **3** and **4** at the central N atom of the seven-membered ring system was achieved by formation of the dilithium salt with *n*-butyllithium and subsequent reaction with the appropriate (dialkylamino)alkyl halide to give compounds **5** and **6**.

Results and Discussion

Nine compounds have been selected for analysis, comprising three different tricycles and three different side chains. The compounds were tested for their affinity to the imipramine and muscarinic receptors in homogenized rat cortex tissue. The results of these studies are shown in Figure 1. Binding affinities are expressed as K_i values. Besides pirenzepine (**1**), all compounds of this study were shown to be nonselective antimuscarinics with Hill coefficients $n = 1$. The pK_a values of the aliphatic amino groups in the piperazine ring of pirenzepine have been found to be 2.05 (N1) and 8.2 (N4).[19,20] Thus, at physiological pH the degree of protonation is only controlled by pK_a 8.2, indicating that the monoprotonated form of pirenzepine present in solution at pH 7.4 exceeds more than 85%.

At pH 7.4 pirenzepine has a log P_{app} of −0.6,[13] in contrast to imipramine with a log P_{app} of 2.4. Thus, just from a comparison of the global lipophilicity of these two species, it becomes evident that hydrophilicity might be a major determinant for the selective binding affinity of pirenze-

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pine toward the M receptor in comparison to imipramine (Figure 1).

However, a detailed knowledge about the influence of structural features on binding affinity and selectivity can be obtained from the result of binding studies summarized in Figure 1.

Analysis of column A reveals that imipramine (**2**) exhibits binding affinities of the same order of magnitude toward the imipramine receptor and the M receptor. The replacement of the CH_2CH_2 group in the central ring by an amide group leads to a 400-fold decrease in binding affinity toward the imipramine receptor whereas the affinity to the M receptor remains unaffected. On the other hand, compounds **5** and **6** show the same selectivity ratio, which means that the introduction of a nitrogen atom into one of the benzene rings has no additional influence on selectivity, even though there is an approximately fourfold decrease in affinity.

Analysis of column B reveals interesting aspects with regard to the influence of the exocyclic amide group on receptor affinity and selectivity. Whereas compound **12** exhibits similar binding affinities for both receptor systems, the introduction of the endocyclic amide group (**9**) leads to a 40-fold increase for the M receptor but decreases the affinity for the imipramine receptor dramatically. In analogy to column A, the change from the dibenzo to the pyrido–benzo system (**9**, **10**) is accompanied by a slight reduction of the binding affinity without effect on selectivity. Analysis of column C indicates that the attachment of the (4-methyl-1-piperazinyl)acetyl side chain to the tricycle (**14**) leads to a system with high M selectivity but rather low affinity. Finally, compound **16** as well as pirenzepine (**1**) containing both the endo- and the exocyclic amide groups exhibit ideal M selectivity in combination with high affinity for the M receptor.

Analysis of Figure 1 in respect to a variation of the side chain again shows interesting features. Replacement of (dimethylamino)propyl side chain of imipramine (**2**) by a (dimethylamino)propionyl system alone does not provide a differentiation of the binding affinities toward the M and imipramine receptors. As indicated already for compound **14**, the introduction of the pirenzepine side chain leads to a very pronounced selectivity but relatively low binding affinity.

An analysis of rows E and F accentuates the large extent to which the endocyclic amide group contributes to high selectivity in combination with high potency toward the M receptor. In comparison, the selectivity effect of the exocyclic amide moiety is not very pronounced.

We assume that the binding of imipramine and pirenzepine and its congeners indeed constitutes an interaction that is highly specific. The lack of binding affinity of pirenzepine for the imipramine receptor therefore reflects major differences in shape and geometry of the binding sites of the muscarinic and imipramine receptors, respectively.

In Figure 2, a putative model has been established that tries to outline important differences between both receptor systems. The 3D shape of pirenzepine shown in Figure 2 corresponds to one out of five energetically favorable conformations one of which could conceivably represent the "active conformation" of pirenzepine.[10] As pirenzepine and imipramine bind to the muscarinic receptor with approximately the same binding affinity, we assume that there is a common structural basis for the binding process of these two species. According to the classical view,[18] the model in Figure 2 describes the accommodation of pirenzepine by way of a three-point at-

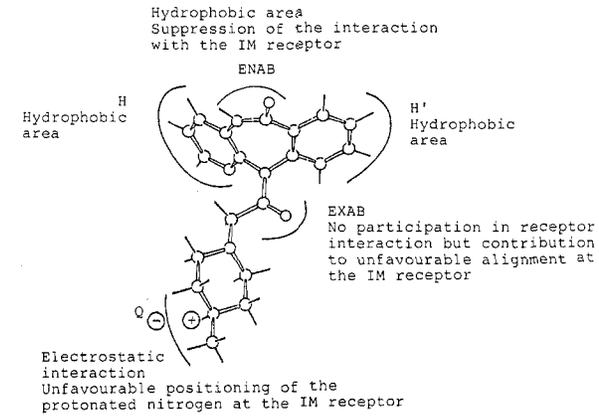

Figure 2. Putative model for the explanation of selective binding of pirenzepine to the muscarinic receptor. ENAB = endocyclic amide group; EXAB = exocyclic amide group; H, H′ = hydrophobic binding area; Q = electrostatic interaction.

tachment (H, H′, Q) via the aromatic rings of the tricycle (H, H′) capable of forming hydrophobic interactions with the receptor surface and the protonated nitrogen of the side chain (Q), which binds via Coulombic attraction to a negative charge on the receptor site. In view of the topographical similarities between both molecules, it would thus appear that the tricycle and the protonated amino function are the crucial determinants for the binding of imipramine to the muscarinic receptor as well. The fact that the distance from the protonated nitrogen to the center of the nearest phenyl ring amounts to 5.0–5.5 Å for imipramine[10] and is much shorter than that for the energetically favored conformations of pirenzepine[10] (6–7 Å) favors the idea that the muscarinic receptor offers several possibilities for the accommodation of antagonists with different spatial geometries. This might be accomplished either by a certain flexibility of the receptor binding site or by a different alignment of the ligand to the receptor.

On the other hand, the lack of binding affinity of pirenzepine for the imipramine receptor suggests major differences in the shape and geometry between the imipramine and the muscarinic receptor.

The structure–binding affinity relationship found for pirenzepine and its congeners clearly indicates that it is the nature and placement of accessory groups that determine the differences in receptor recognition and the binding process. In the case of pirenzepine, the preferential binding to the M receptor is brought about by three different features, namely, by the endocyclic amide group, by the position of the protonated nitrogen in respect to the tricyclic ring system, and to a minor extent by the exocyclic amide bond.

The suppressive effect of the endocyclic amide group (Figure 2, ENAB) with respect to the interaction with the IM receptor can be rationalized by the assumption that parts of the "back wall" of the IM binding site consist of a hydrophobic area which in contrast to the corresponding area of the M receptor does not participate in a polar binding process such as hydrogen bonding. This site at the IM receptor is most probably a lipophilic cavity and hence does not interact with the endocyclic carboxamide group of pirenzepine. On the other hand, this hydrophobic cavity enforces the binding affinity of imipramine via hydrophobic interaction.

The presence of the exocyclic amide group (Figure 2, EXAB) seems to be of minor influence for favorable binding to the M receptor. It can be concluded that this group does not participate in receptor interaction but inhibits a favorable alignment to the IM receptor. This effect

seems to be enhanced by the influence of the (4-methyl-1-piperazinyl)acetyl side chain, indicating that the complementary negative charge at the IM receptor site (Figure 2, Q) lies in a position that cannot be favorably accommodated by the protonated N atom of pirenzepine.

Experimental Section

Melting points were determined in Pyrex capillaries on a Büchi 510 melting point apparatus and are uncorrected. Microanalyses were performed by the Thomae Research Microanalysis Laboratory. ^{1}H NMR spectra were recorded on a WP 80 Bruker spectrometer; chemical shifts are reported with reference to internal tetramethylsilane. IR and NMR spectra were consistent with assigned structures for all compounds. Silica gel was used for chromatography.

Imipramine has been purchased from Aldrich. Pirenzepine was provided by the Thomae Co. Compounds **9**,[14] **12**,[15] and **13**[15] were prepared according to known methods from the literature.

5,10-Dihydro-5-[3-(dimethylamino)propyl]-11*H*-dibenzo-[*b*,*e*][1,4]diazepin-11-one (5). *n*-Butyllithium (76 mL of a 1.5 M solution in hexane, 0.115 mol) was added to a suspension of **3**[16] (8.4 g, 0.04 mol) in dioxane (200 mL) at room temperature. The mixture was stirred for 2 h and, after addition of 3-(dimethylamino)propyl chloride (6 g, 0.05 mol), heated under reflux for 17 h. The solvent was removed in vacuo and the residue treated with diluted hydrochloric acid whereupon a solid precipitated. After filtration the residue was washed twice with CH_2Cl_2 and dissolved in water. The aqueous solution was made alkaline by the addition of K_2CO_3 and extracted twice with CH_2Cl_2. The organic layer was separated, and the solvents were dried with Na_2SO_4 and evaporated under reduced pressure. The residue was eluted through a silica gel column using a mobile phase of CH_2Cl_2/cyclohexane/CH_3OH/aqueous NH_3 (102:23:23:3). The solvents were removed from the main fraction to yield 4.5 g (38%) of product **5**: mp 143–146 °C (acetonitrile/ethyl acetate); ^{1}H NMR (CD_3OD, $CDCl_3$) δ 7.0–7.8 (m, 8 H), 3.7 (t, 2 H), 2.4 (t, 2 H), 2.2 (s, 6 H), 1.7 (m, 2 H); IR (CH_2Cl_2) 3380, 1660 cm^{-1}. Anal. ($C_{18}H_{21}N_3O$) C, H, N.

5,11-Dihydro-11-[3-(dimethylamino)propyl]-6*H*-pyrido-[2,3-*b*][1,4]benzodiazepin-6-one (6). This compound was prepared by following the procedure described for compound **5**. From **4**[17] (6 g, 0.03 mol), *n*-butyllithium (57 mL of a 1.5 M solution in hexane, 0.086 mol), and 3-(dimethylamino)propyl chloride (4.5 g, 0.037 mol), compound **6** was obtained after recrystallization from ethyl acetate/acetonitrile in a yield of 2.7 g (23%): mp 135–136 °C; ^{1}H NMR (CD_3OD/$CDCl_3$) δ 6.9–8.1 (m, 8 H), 3.9 (t, 3 H), 2.3 (t, 2 H), 2.1 (s, 6 H), 1.7 (m, 2 H); IR (KBr) 3300, 3170, 1660 cm^{-1}. Anal. ($C_{17}H_{20}N_4O$) C, H, N.

11-(3-Chloro-1-oxopropyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (8). 3-Chloropropionyl chloride (56 mL, 0.44 mol) dissolved in dioxane (144 mL) and a mixture of triethylamine (74 mL) and dioxane (126 mL) were simultaneously added to a solution of compound **4**[17] (84 g, 0.4 mol) dissolved in dioxane at 100 °C. The reaction mixture was stirred under reflux for 4 h. After cooling, the precipitate was removed by filtration and the solvents were evaporated in vacuo to afford a white solid. Recrystallization from ethanol afforded 30 g (30%) of **8**, mp 235–240 °C.

5,11-Dihydro-11-[3-(dimethylamino)-1-oxopropyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (10). A mixture consisting of compound **8** (9.0 g, 0.03 mol), ethanol (250 mL), and dimethylamine (2.7 g, 0.06 mol) was heated in an autoclave at 100 °C for 5 h. The reaction mixture was cooled and the solvent removed in vacuo. The resulting viscous residue was purified by column chromatography (silica gel, CH_2Cl_2/cyclohexane/CH_3OH/aqueous NH_3, 102:23:23:3) and the main fraction recrystallized from ethanol to afford 5.0 g (54%) of **10**: mp 193–194 °C; ^{1}H NMR (CD_3OD/$CDCl_3$) δ 7.3–8.4 (m, 8 H), 2.7 (m, 4 H), 2.2 (s, 6 H); IR (KBr) 3370, 1685, 1665 cm^{-1}. Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

5-[(4-Methyl-1-piperazinyl)acetyl]-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one (14). A mixture of compound **13**[14] (4.3 g, 0.015 mol), *N*-methylpiperazine (10 mL, 0.09 mol), and dioxane (200 mL) was heated under reflux for 18 h. Evaporation of the solvent gave a crude product, which was made alkaline with diluted aqueous NaOH. The mixture was extracted into CH_2Cl_2, and the organic layer was washed with water and dried (Na_2SO_4). Removal of the solvent, chromatography (silica gel, CH_3OH/aqueous NH_3, 20:1), and recrystallization (2-propanol/ether) afforded 1.7 g (32%) of **14** as white crystals, mp 126–127 °C. Anal. ($C_{20}H_{22}N_4O_2$) C, H, N.

5-(Chloroacetyl)-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine (15). Compound **11** (10.0 g, 0.051 mol) and chloroacetyl chloride (11.3 g, 0.1 mol) in toluene (50 mL) were heated under reflux for 5 h. After removal of the solvent in vacuo, the residue was recrystallized from ethyl acetate, affording 7.4 g (53%) of **15**, mp 95–96 °C.

5-[(4-Methyl-1-piperazinyl)acetyl]-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine (16). Compound **15** (7.4 g, 0.027 mol), *N*-methylpiperazine (4.5 g, 0.045 mol), and Na_2CO_3 (4.8 g, 0.045 mol) in ethanol (75 mL) were heated under reflux for 5 h. After evaporation to dryness, a white residue was obtained, which afforded 7.6 g (84%) of compound **16** after recrystallization from cyclohexane, mp 98–99 °C. IR (KBr) 2700–2100, 1675 cm^{-1}. Anal. ($C_{21}H_{25}N_3O$) C, H, N.

Biochemistry. Imipramine Binding Assay. Male rats (strain Thom. CHBS) of ca. 200 g body weight were killed by a blow on the neck. The animals were decapitated, and the forebrain was carefully prepared. This cortical tissue was weighed and immersed in 60 mL of ice cold buffer (Tris·HCl, 50 mM; NaCl, 100 mM; KCl, 5 mM; pH 7.5) and was homogenized by an Ultra Turrax for 1 min. The homogenate was sedimented for 10 min at 50000*g* following two washings. The final pellet was resuspended in 60 mL of buffer and homogenized by a glass–Teflon homogenizer. The final tissue dilution was 1:250.

The compounds were diluted together with 2 nM [^{3}H]imipramine (specific activity 2.84 TBeq/mmol, purchased from NEN) in 2 mL of chilled buffer in test tubes and placed on ice. The incubation at 0° C was started by addition of 2 mL of membrane preparation and was terminated after 1 h by rapid filtration through presoaked Whatman GF/B glass fiber filters under vacuum. The vials and filters were rinsed three times by 5 mL of ice cold buffer. The filters were transferred to counting vials, extracted overnight with 10 mL of Instagel, and counted for radioactivity in a Packard Tri-Carb 480 CD liquid-scintillation counter at 50% efficiency. The specific binding was defined as the amount of total binding displaceable by 100 μM desipramine.

Muscarinic Binding Assay. The forebrain used in this binding assay was prepared as described above. It was homogenized in 70 mL of buffer (HEPES, 20 mM; NaCl, 100 mM; $MgCl_2$, 10 mM; pH 7.5) with an Ultra Turrax for 1 min. The final tissue dilution was 1:3000.

The radioactive ligand [^{3}H]-*N*-methylscopolamine ([^{3}H]NMS, specific radioactivity 3.145 TBeq/mmol, purchased from NEN) was placed at a concentration of 0.3 nM together with the test compound in a volume of 80 μL in small centrifuge tubes. The incubation was started by addition of 1 mL of homogenate and was run at 30 °C for 30 min. The incubation was terminated by rapid centrifugation. The pellets were rinsed twice with 2 mL of saline and dissolved overnight in 100 μL of Soluene. Thereafter 1.7 mL of Dimilume scintillation fluid was added and the samples were counted for radioactivity. Specific binding was measured by addition of 1 μM 3-quinuclidinyl benzoate ((−)-QNB).

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