adjusted to pH 7.4 at 37 °C. In this medium, the cells have a generation time of 24–30 h and remain in the log phase of growth between a cell density of (2–8) × 10^5 cells/mL. All incubations were carried out in spinner flasks at 37 °C under magnetic stirring (100 rpm). Test steroids were added in a 10 μL solution of ethanol per 10 mL of culture (density 2 × 10^5 cells/mL). Equivalent amounts of ethanol were added to control samples. Cell viability was tested by trypan blue exclusion. To assay TAT, 2-mL samples of the cell culture were removed, and the cells were collected by centrifugation. The pellets were washed twice in isotonic saline and thereafter suspended and chilled in 1 mL of 5 × 10^{-2} M potassium phosphate buffer (pH 7.6), 2 × 10^{-3} M 2-oxoglutarate, 1 × 10^{-4} M pyridoxal phosphate, and 1 × 10^{-3} M EDTA. The cells were disrupted with an ultrasonicator (250 TS-20K, Annemasse, France) by two consecutive exposures to 70 V for 20-s bursts. The enzyme was assayed at 37 °C by the method of Diamondstone.[71] One unit of activity represents the formation of 1 μmol of *p*-hydroxyphenylpyruvate/minute. Enzyme-specific activity is expressed as milliunits of TAT/milligram of cell protein. The protein content was measured by the method of Lowry et al. with BSA as standard. The maximum steady-state enzymic activity obtained with 10^{-6} M dexamethasone (70–80 milliunits/mg of protein) corresponds to 100% in Table I.

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Acknowledgment. The studies reported in this paper on glucocorticoid activity were performed with the collaboration of G. Beck (CNRS, Strasbourg). The relative binding affinities were determined with the invaluable technical assistance of M. Fortin (Roussel-Uclaf). We thank C. Marchandeau for his helpful comments on the manuscript.

Registry No. 1, 65049-45-8; **2**, 382-67-2; **3**, 76-25-5; 4, 67-73-2; **5**, 67983-60-2; **6**, 67983-56-6; **7**, 67983-73-7; **8**, 50-02-2; **9**, 378-44-9; **10**, 426-15-3; 11, 16320-04-0; **12**, 65049-43-6; **13**, 50-22-6; 14, 10417-63-7; **15**, 64-85-7; **16**, 1816-78-0; **17**, 4906-84-7; **18**, 124-94-7; **19**, 124-94-7; **20**, 50-23-7; **21**, 1239-79-8; **22**, 34184-77-5; **23**, 1177-87-3; **24**, 10116-22-0; **25**, 52-39-1; **26**, 152-58-9; **27**, 57-83-0; **28**, 50-03-3; **29**, 74183-64-5; **30**, 4004-68-6; **31**, 50-28-2; **32**, 58-22-0; **33**, 64313-94-6; **34**, 53-06-5; **35**, 68-22-4; **36**, 14531-92-1; **37**, 88256-64-8; **38**, 88256-65-9; **39**, 84371-65-3; TAT, 9014-55-5.

Supplementary Material Available: Details of correspondence analysis, i.e., coordinates and relative and absolute contributions (3 pages). Ordering information is given on any current masthead page.

Tricyclic Compounds as Selective Antimuscarinics. 2. Structure–Activity Relationships of M_1-Selective Antimuscarinics Related to Pirenzepine

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In order to gain some insight into those structural features that control M_1 selectivity, a selected set of pirenzepine analogues has been studied in which both the tricyclic ring system and the basic side chain have been varied. Binding studies were conducted in rat tissue homogenates from cerebral cortex (M_1) and gastric fundus (M_2). The ratio of IC_{50} values of the test compounds in the two different tissues was taken as a measure of M_1 receptor selectivity. Several derivatives, especially those with flexible side chains, i.e. high degree of freedom of rotation around single bonds, proved to be nonselective. Among semirigid compounds only those containing 6-membered ring systems (11, 13, 14, and 15) showed significant M_1 selectivity. Principles of structure–activity and structure–selectivity are discussed.

Pirenzepine (**2**) is a tricyclic drug that unlike psychotropic tricyclic agents exhibits measurable inhibitory effects exclusively toward the muscarinic receptor system.[1] The underlying structural requirements for selectivity toward the muscarinic acetylcholine receptor have been discussed in a preceding paper.[2] Pirenzepine (**2**) has been introduced into ulcer therapy, providing safe and unproblematic treatment of gastritis and duodenal ulcer.[3] Both experimental and clinical evidence point to the fact that the therapeutic effects of pirenzepine are due to a selective blockade of M_1 receptors governing gastric secretion.[4] In humans, significant reduction of gastric acid and pepsinogen secretion is obtained at plasma levels at which other antimuscarinic effects, like mydriasis, inhibition of gastric emptying, inhibition of salivation, and impairment of esophageal motility, do not occur. Moreover, tachycardia, a common side effect of classical antimuscarinics, is not observed following pirenzepine treatment.[5]

Receptor-binding studies have provided the first insight into the mode of action of pirenzepine (**2**). It has been shown that this compound is able to discriminate between high- and low-affinity binding sites within the muscarinic acetylcholine receptor system. Outside the central nervous system, the high-affinity subtype appears to be prevalent in the sympathetic and myenteric ganglia whereas low-affinity subtypes are found in high proportions in peripheral muscarinic effector organs such as heart, exocrine glands, and smooth muscle.[6] An evolving classification scheme for these muscarinic receptors divides them into M_1 (high affinity) and M_2 (low affinity) subtypes. Although details of the distinctions are still in the beginning, this characterization offers a sound basis on which to compare the binding properties of different muscarinic antagonists and to assess their potential M_1 selectivity.

As part of our interest in the development of agents that selectively interact with the muscarinic system, we have

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‖ Deceased.

(1) Bechtel, W. D.; Mierau, J.; Pelzer, H. *Arzneim.-Forsch./Drug Res.* **1986**, *36*, 793.
(2) Eberlein, W. G.; Trummlitz, G.; Engel, W. W.; Schmidt, G.; Pelzer, H.; Mayer, N. *J. Med. Chem.* **1987**, *30*, 1378.
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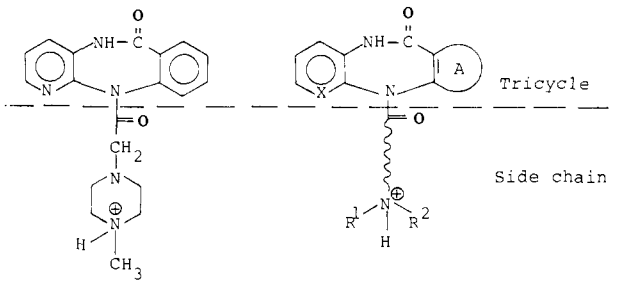
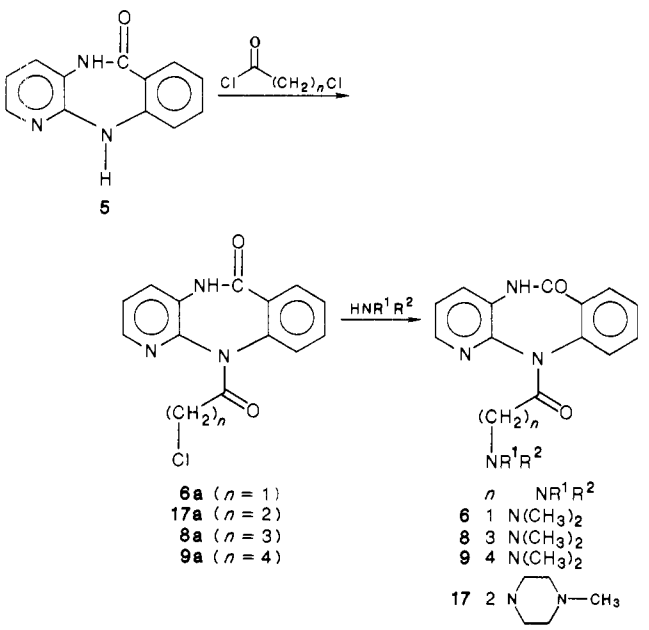


Figure 1. Pirenzepine (**2**) and type of structural modification with respect to tricycle and side chain.

Scheme I



undertaken the design, synthesis, and biological evaluation of a variety of pirenzepine analogues in order to identify those molecular parameters that control M_1 selectivity (Figure 1).

Binding Studies

Binding studies were conducted in rat tissue homogenates.[8] The nonselective radioligand [^{3}H]-*N*-methylscopolamine at a concentration of 0.3 nM was used to label muscarinic receptors in preparations of cerebral cortex and gastric fundus containing preferentially M_1 and M_2 receptors, respectively.

IC_{50} values (nM) for binding affinity to muscarinic receptors in the cortex were taken as estimates for potency. The ratio of IC_{50} values of test compounds in the two different tissues, i.e.

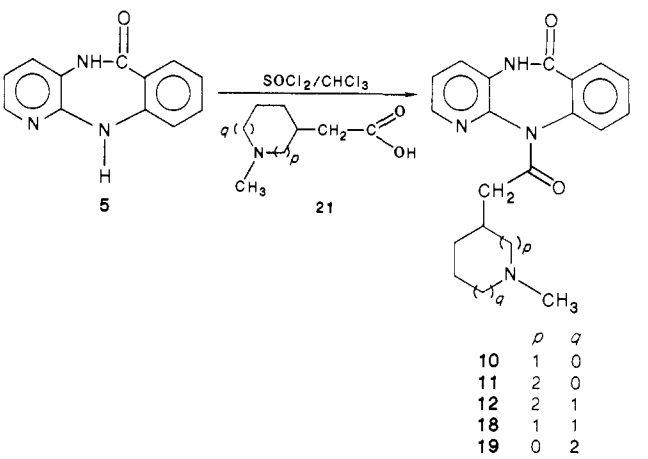
$$\frac{IC_{50}\ \text{fundus (nM)}}{IC_{50}\ \text{cortex (nM)}} = \frac{F(M_2)}{C(M_1)}$$

was taken as a measure of M_1 receptor selectivity.

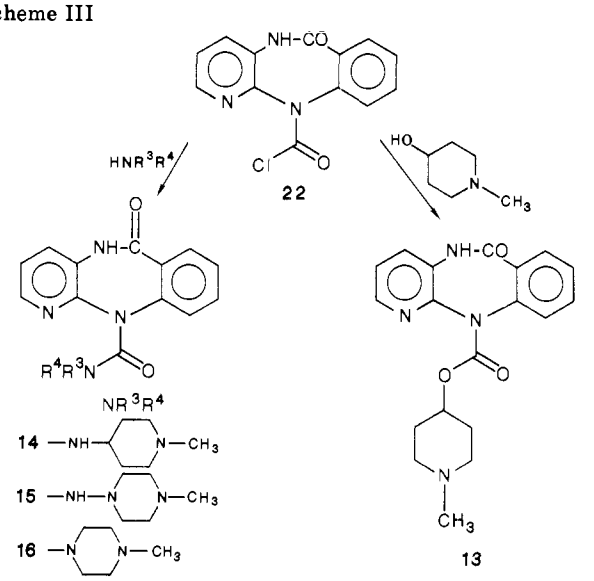
Chemistry

Table I lists the formula and physical data of the new compounds that were prepared via synthetic methodology described already in the literature. Acylation of tricycle **5** was readily effected by reaction with the corresponding

Scheme II



Scheme III



chloroacyl halides leading to the intermediate ω-chloroacyl amides **6a**, **8a**, **9a**, and **17a**, which in turn by reaction with secondary amines furnished the expected basic tricycles **6**, **8**, **9**, and **17** (Scheme I). Compounds **10**–**12**, **18**, and **19** were obtained as indicated in Scheme II by reaction of **5** with the corresponding acyl chlorides of type **21** with chloroform as a solvent. Reaction of the (chlorocarbonyl)pyridobenzodiazepinone **22** with amines or secondary amino alcohols as indicated in Scheme III led to the desired compounds **14**, **15**, **16**, and **13**, respectively.

Biological Results and Discussion

Biological data of substituted tricyclic diazepinones are recorded in Table I. The influence of the tricyclic system on binding affinity and M_1 selectivity can be demonstrated by a comparison of compounds **1**–**4**. It can be seen that the binding affinity to the muscarinic acetylcholine receptors is strongly dependent on the nature of the heterocyclic ring system and varies for example in case of fundus tissue up to 30-fold. In order to rationalize these results, different aspects have to be considered. First, it has been shown by molecular mechanics calculations[10] that differences in the spatial geometry of different tricyclic ring systems are only marginal, and hence, differences in

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(8) Hammer, R.; Berrie, C. P.; Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. *Nature (London)* **1980**, *283*, 90–92.
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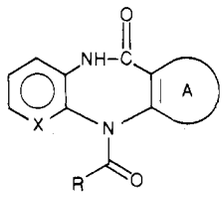
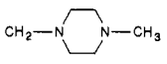
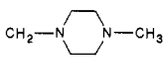
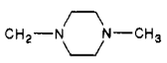
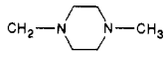
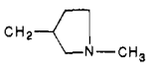
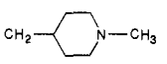
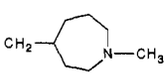
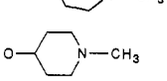
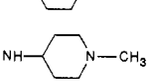
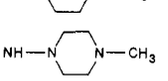
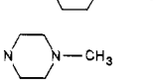
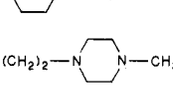
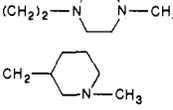
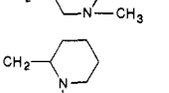
Table I. Physical Properties and Binding Assays of Substituted Tricyclic Diazepinones



no.	X	A	R	recrystn solvent	mp, °C	yield,[a]	mol[b] formula	IC_{50},[c] nM cortex	IC_{50},[c] nM fundus	F/C[d]
1	CH			2-propanol/ether	126–127	32	$C_{20}H_{22}N_4O_2$	23	100	~4
2	N			2-propanol	250–251 dec		$C_{19}H_{23}Cl_2N_5O_2$	120	1200	10
3	CH			acetone	177–178	60	$C_{18}H_{20}N_4SO_2$	50	350	7
4	CH			2-propanol/ether	232–234	79	$C_{19}H_{21}N_5O_2$	300	3000	10
6	N		$CH_2N(CH_3)_2$	ethyl acetate	195–197	67	$C_{16}H_{16}N_4O_2$	1500	700	0.5
7	N		$(CH_2)_2N(CH_3)_2$	acetonitrile	193–194	54	$C_{17}H_{18}N_4O_2$	200	200	1
8	N		$(CH_2)_3N(CH_3)_2$	acetonitrile	207–209	36	$C_{18}H_{20}N_4O_2 \cdot HCl$	50	100	2
9	N		$(CH_2)_4N(CH_3)_2$	acetonitrile	152–153	25	$C_{19}H_{22}N_4O_2$	600	500	1
10	N			ethyl acetate	178–179	10	$C_{19}H_{20}N_4O_2$	20	50	2.5
11	N			ethyl acetate/methanol	237–239	23	$C_{20}H_{22}N_4O_2$	70	700	10
12	N			ether	158–160	11	$C_{21}H_{24}N_4O_2$	100	150	1.5
13	N			ethyl acetate	247–248	77	$C_{19}H_{20}N_4O_3$	100	900	9
14	N			ethanol	144–147	63	$C_{19}H_{21}N_5O_2$	200	1200	6
15	N			ethyl acetate	201–203 dec	17	$C_{18}H_{20}N_6O_2$	60	900	15
16	N			acetonitrile	230–233	28	$C_{18}H_{19}N_5O_2$	150	600	4
17	N			ethanol	224–228	15	$C_{20}H_{23}N_5O_2$	10000	12000	1
18	N			ethyl acetate	208–209	21	$C_{20}H_{22}N_4O_2$	100	120	1
19	N			ethyl acetate	172–173	14	$C_{20}H_{22}N_4O_2$	10	8	1

[a] No attempt was made to optimize yields. [b] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values. [c] All IC_{50} values were obtained in primary screening; no correction has been made for compounds exhibiting Hill coefficients (n_H) ≤ 1. The reported values represent single experiments performed in quadruplicate. [d] *N*-Methylscopolamine has been used as reference compound with the following IC_{50} (nM) values: cortex 0.72, stomach wall 1.34, $F/C \sim 1$–2.

binding affinities as indicated in Table I cannot be explained by different folding of the tricyclic ring of these molecules. Moreover if one assumes that pirenzepine is bound to the M receptor via a threepoint attachment,[2] one can expect that binding will occur preferentially with lipophilic tricyclic systems. On the other hand, tricycles with reduced hydrophobicity as brought about by substitution of ring carbon atoms by nitrogen atoms should exhibit lower binding affinities. This view is in line with the finding that decreases of affinity parallel those of hydrophobic properties as expressed by log P_{app} at pH 7.4 (Table II). The influence of different tricycles on selectivity is moderate as all the compounds in the above-mentioned series exhibit a tendency toward M_1 selectivity with the pyridobenzodiazepinones **2** and **4** showing highest selectivity.

Table II. Partition Coefficients

compd	log P_{app}[a] at pH 7.4
1	0.11
3	−0.15
4	−0.42
2	−0.64[7]

[a] Partition coefficient log P_{app} was determined between 1-octanol and an aqueous buffer solution at pH 7.4 (25 °C).

A comparison of compounds **6–12** demonstrates the influence of the side chain on M-receptor affinity and selectivity. Within this series, compounds **6–9** represent a set of pirenzepine analogues with highly flexible side chains. These systems have a large number of energetically favored conformations due to high degree of freedom of rotation about single bonds. It can be recognized that the chain length as indicated by the number of CH_2 increments plays a crucial role in M binding affinity. Maximal affinity is provided by n = 2–3, indicating thus an optimal fit to the receptor. As shown in Table I, M_1 selectivity is not obtained in the case of systems with flexible side chains. Apparently such systems can easily adapt to different structural environments at the receptor and can interact therefore with different subclasses of muscarinic receptors that differ with respect to their conformations. This hypothesis would favor the idea that reduction of conformational freedom should increase the probability of M_1 selectivity. Pirenzepine analogues with a certain conformational constraint of the side chain are represented by compounds **10–12**. In this case the principles of semirigidity are brought about by the remaining degrees of conformational freedom, i.e., limited rotation about two single bonds. It is interesting to note that although compounds **10–12** exhibit quite similar affinities to the M_1 receptor, only the 6-membered ring derivative **11** shows pronounced selectivity. This lack of M_1 selectivity of compounds **10** and **12** could be due to conformational (flexibility or spacial alignment) differences. It is well known that 5- and 7-membered ring systems have considerably more conformational freedom within their steric limits in comparison to 6-membered ring systems.[11,12] For example, it has been shown for pyrrolidine[13] that the envelope and half-chair conformers populate a pseudorotational circuit with small energy differences between individual conformers (0–1.0 kcal/mol) and are therefore without well-marked energy minima and maxima. Similarly, molecular mechanics calculations of disubstituted pyrrolidine and azepine rings show that at least half of the possible conformers of the whole pseudorotational circuit differ in their conformational energy by less than 0.5 kcal.[14] It can be anticipated therefore that the 5- and 7-membered ring systems are less sensitive to the conformational differences between subclasses of different muscarinic receptors; consequently poor M_1 selectivity is observed.

Compounds **2**, **11**, **13**, **14**, and **15** represent a series of bioisosteric 6-membered ring systems. In accordance with the view that M_1 selectivity can be brought about by 6-membered ring systems, those compounds exhibit pronounced M_1 selectivity with an approximate selectivity ratio F/C of 10. The principles of bioisosterism refer both to selectivity as well as to binding affinity. It can be seen that certain atoms or groups within the (4-methyl-1-piperazinyl)acetyl side chain of pirenzepine can be replaced without causing large changes in binding affinity or selectivity. Thus a CH_2 group can be replaced by an oxygen atom or by a NH group and a nitrogen atom can be substituted by a CH group. Interestingly the exchange of CH_2 by a NH group leads to the hydrazino compound **15** exhibiting high binding affinity and very pronounced M_1 selectivity.

A comparison of compounds **11**, **18**, and **19** outlines the influence of the position of the basic N atom in the 6-membered rings. A prerequisite for the achievement of M_1 selectivity seems to be the appropriate spatial location of the protonated side chain nitrogen atom relative to the tricyclic backbone. Though high binding affinity toward the muscarinic receptors is retained for compounds **18** and **19**, neither of these two species exhibits M_1 selectivity.

Finally, the influence of the length of the methylene chain $-(CH_2)_n-$ that connects the pyridobenzodiazepine tricycle with the 4-methyl-piperazine ring is demonstrated by a comparison of compounds **2**, **16**, and **17**. An increase in flexibility by extending the chain length by one methylene unit (n = 2) results in complete loss of selectivity and a sharp drop in binding affinity. Compound **16** on the other hand, which is characterized by a strong conformational constraint of the side chain, retains both affinity as well as a tendency toward M_1 selectivity.

Summarizing the results obtained from qualitative structure–activity and structure–selectivity analysis, one can draw the following conclusions.

M-Receptor Affinity. The M-receptor affinity of pirenzepine and its analogues is controlled by both the tricycle and the side chain. One determining factor for the influence of the tricycle on binding affinity seems to be the global lipophilicity of the individual systems.

M_1 Selectivity. M_1 selectivity is controlled by both the tricyclic system and the side chain but with dominant influence of the side chain. In contrast to M affinity it seems that M_1 selectivity is independent of global lipophilicity. Among the tricycles studied, highest selectivity has been obtained with compounds containing as a tricycle the pyridobenzodiazepinone system.

As to the influence of the side chain on selectivity, we can state that compounds containing flexible side chains with extensive conformational freedom about single bonds do not exhibit M_1 selectivity.

Among semirigid systems only 6-membered ring systems have been found to exhibit reasonable selectivity with F/C = 5–15.

The lack of M_1 selectivity for 5- and 7-membered ring systems can be either due to a change in the spatial position of the protonated nitrogen atom in relation to the tricyclic backbone or to the phenomena of pseudorotational flexibility.

The consistency among bioisosteric 6-membered ring systems in regard to selectivity and M affinity underlines the importance of exact spatial orientation of the side chain N atom relative to the tricyclic backbone.

Further computational studies (conformation space analysis) directed toward a refined understanding of the preferred receptor-bound conformations in this class of compounds have been described recently.[23]

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Experimental Section

Melting points were determined in open Pyrex glass capillaries on a Büchi 510 melting point apparatus and are uncorrected. Microanalyses were performed by the Thomae Research Microanalysis Laboratory and were correct within ±0.4% of the theoretical values. ^{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.

Pirenzepine (**2**) has been provided by the Thomae Co. Compounds **1**,[15] **3**,[16] **4**,[17] and **7**[2] were synthesized according to known procedures from the literature.

5,11-Dihydro-11-[2-(dimethylamino)-1-oxoethyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (6). A solution of **6a**[19] (8.0 g, 0.028 mol) in acetone (60 mL) and 40% aqueous dimethylamine (100 mL) were stirred at room temperature for 2 h and evaporated. The oily residue was dissolved in dilute acetic acid and extracted with 2 × 50 mL of chloroform. The aqueous portions were treated with charcoal and after filtration made alkaline by the addition of concentrated ammonia. The resulting oil was extracted twice with chloroform. The combined chloroform portions were dried (Na_2SO_4) and evaporated. Recrystallization from ethyl acetate afforded 5.5 g (67%) of **6**, mp 195–197 °C. Anal. ($C_{16}H_{16}N_4O_2$) C, H, N.

5,11-Dihydro-11-[4-(dimethylamino)-1-oxobutyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (8). A solution of compound **8a**[20] (3.1 g, 0.01 mol) in ethanol (150 mL) and dimethylamine (1.5 g) was heated in an autoclave at 100 °C for 5 h. Evaporation of the solvent resulted in a glassy brown residue, which was purified by column chromatography (silica gel, CH_2Cl_2/cyclohexane/CH_3OH/aqueous NH_3, 102:23:23:3). The main fraction was dissolved in ether, treated with an ethereal solution of hydrogen chloride, and recrystallized from acetonitrile, yielding 1.3 g (36%) of **8**, mp 207–209 °C, as white crystals. Anal. ($C_{18}H_{20}N_4O_2$·HCl) C, H, N, Cl.

5,11-Dihydro-11-[5-(dimethylamino)-1-oxopentyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (9). This compound was prepared by following the procedure described for compound **8** by reaction of **9a**[20] (9.8 g, 0.030 mol) in 200 mL of ethanol with aqueous dimethylamine (2.7 g, 0.045 mol) at 100 °C in an autoclave for 5 h. Purification by column chromatography (silica gel, CH_2Cl_2/cyclohexane/CH_3OH/aqueous NH_3, 102:23:23:3) followed by recrystallization from acetonitrile afforded 2.5 g (25%) of **9**, mp 152–153 °C. Anal. ($C_{19}H_{22}N_4O_2$) C, H, N.

5,11-Dihydro-11-[3-(4-methyl-1-piperazinyl)-1-oxopropyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (17). Compound **17a**[2] (17.0 g, 0.056 mol) and *N*-methylpiperazine (60 mL) in 2-propanol (240 mL) were heated under reflux for 2 h. After treatment with charcoal the solvent was evaporated. The residue was recrystallized from a mixture of ethanol/ethyl acetate, affording 3.0 g (14.6%) of **17**, mp 224–228 °C. Anal. ($C_{20}H_{23}N_5O_2$) C, H, N.

General Procedure for the Preparation of Pyridobenzodiazepinones 10–12, 18, and 19. Thionyl chloride (9.5 g, 0.08 mol) dissolved in chloroform (20 mL) was added dropwise to a suspension of the potassium salt of the corresponding *N*-methylazacycloalkylacetic acid[21] (0.072 mol) in anhydrous chloroform (150 mL) with external cooling and with the reaction temperature being maintained at 15 °C. The mixture was stirred for a further 20 min and then concentrated to dryness in vacuo. The remaining residue was added to a suspension of 5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one[18] (8.4 g, 0.04 mol) in a mixture of solvents consisting of absolute dioxane (300 mL) and anhydrous pyridine (20 mL). The mixture was heated to 80 °C for 2 h with rigorous stirring. After cooling, the mixture was filtered and the residue was taken up in water. It was made alkaline with solid sodium carbonate and the aqueous phase was extracted exhaustively with chloroform. The combined chloroform extracts were dried, and the solvent was removed in vacuo. The residue was purified by column chromatography on alumina with ethyl acetate and methanol (ratio by volume 4:1) as eluent. The isolated substances **10–12**, **18**, and **19** were dried and recrystallized from solvents as indicated in Table I.

General Procedure for the Preparation of Pyridobenzodiazepinones 14 and 16. 11-(Chlorocarbonyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1.4]benzodiazepin-6-one[22] (3.6 g, 0.013 mol) and sodium carbonate (1.49 g, 0.01 mol) were refluxed together with the corresponding secondary or tertiary amines (0.016 mol) for 3 h in anhydrous ethanol (80 mL). The mixture was filtered while hot and the filtrate evacuated in vacuo. The crude compounds were recrystallized from solvents as indicated in Table I.

5,11-Dihydro-11-[[(4-methyl-1-piperazinyl)amino]carbonyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (15). A suspension of 11-(chlorocarbonyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (5.5 g, 0.20 mol), 1-amino-4-methylpiperazine (6.99 g, 0.06 mol), and anhydrous dioxane (150 mL) was heated for 30 min on a steam bath, and the cloudy reaction mixture obtained was then mixed while still hot with 2 g of active charcoal and filtered and the filtrate obtained was concentrated by evaporation in vacuo. The residue was purified by column chromatography on 300 g of silica gel, using a mixture of dichloromethane, methanol, and concentrated aqueous ammonia (ratio by volume 800:200:5). The residue remaining after the eluates of interest had been evaporated was recrystallized from ethanol. Colorless crystals of 5,11-dihydro-11-[[(4-methyl-1-piperazinyl)amino]carbonyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one hydrochloride hemihydrate was obtained (1.5 g, 19%), mp 218–219 °C. The salt was dissolved in water, made alkaline with the calculated quantity of an aqueous 10% sodium hydroxide solution, and concentrated by evaporation in vacuo at a bath temperature of 40 °C. The residue remaining was taken up in dry tetrahydrofuran and filtered, and the solvent was again removed from the filtrate in vacuo. After recrystallization from ethyl acetate, the desired water-soluble, colorless base was obtained as colorless crystals: mp 201–203 °C; ^{1}H NMR ($CDCl_3$) δ 8.2 (dd, 1 H), 7.85 (dd, 1 H), 7.7–7.1 (m, 5 H), 6.8 (s, 1 H), 2.6–2.9 (m, 4 H), 2.0–2.6 (m, 8 H); IR (CH_2Cl_2) 1690, 1670 cm^{-1}. Anal. ($C_{18}H_{20}N_6O_2$) C, H, N.

5,11-Dihydro-11-[[(1-methyl-4-piperidinyl)oxy]carbonyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (13). 11-(Chlorocarbonyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (4.1 g, 0.015 mol) and 1-methyl-4-piperidinol (3.45 g, 0.03 mol) were dissolved in chlorobenzene (50 mL) and refluxed for 2 h. After cooling, the reaction mixture was diluted by the addition of ethyl acetate (200 mL) and then extracted exhaustively with 15% hydrochloric acid. The combined extracts were neutralized with potassium carbonate and then extracted several times with chloroform, and the combined chloroform extracts were concentrated to dryness in vacuo. The residue was purified by column chromatography on silica gel with a mixture of ethyl acetate and methanol (ratio of volumes = 1:1) as eluent. Colorless crystals were obtained, mp 247–248 °C, in a yield of 4.0 g (77%): ^{1}H NMR (DMSO) δ 8.3 (dd, 1 H), 7.8 (dd, 1 H), 7.65 (dd, 2 H), 7.5 (m, 3 H), 4.7 (s, 1 H), 2.3 (m, 7 H), 1.75 (m, 2 H), 1.55 (m, 2 H); IR (KBr) 1715, 1665 cm^{-1}. Anal. ($C_{19}H_{20}N_4O_3$) C, H, N.

Biochemistry. Receptor-Binding Assay. All manipulations and assays were performed in 100 mM NaCl, 10 mM $MgCl_2$, and 20 mM HEPES (pH 7.5). Stomach wall was dissected free of mucosa and homogenized (Ultra-Turrax, setting 6.2 × 30 s, 0 °C). The initial homogenate was further homogenized (Potter-Elvehjem, 20 strokes, 520 rpm, 0 °C) and filtered through cheesecloth. It was found that the presence of the proteolytic inhibitor, phenylmethanesulfonyl fluoride (1 mM) had little effect on the receptor-binding properties. The cerebral cortex was only subjected to the Potter homogenization. For the binding assays the homogenates in appropriate dilutions (~1 mg of protein mL^{-1}) were preincubated for 10 min at 30 °C before addition to microcentrifuge tubes containing labeled ligands with or without

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unlabeled ligands. Incubation was carried out for 20 min (at which time, binding was at equilibrium) at 30 °C and was terminated by centrifugation as previously described.[8] Assays were carried out in quadruplicate, and the nonspecific binding was defined as the radioactivity bound or entrapped in the pellet when the incubation medium contained 10^{-6} M 3-quinuclidinyl benzilate.

Partition Coefficients. The partition coefficients were determined between 1-octanol and an aqueous buffer solution at pH 7.4 (20 °C). The determination of concentrations in each phase was performed spectrophotometrically.

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Derivatives of 2-Methylenepropane-1,3-diol as New Antagonists of Platelet Activating Factor

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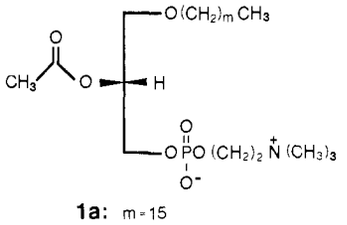
Two new achiral platelet activating factor (PAF) antagonists, *N*-[5-[[2-methylene-3-[[(octadecylamino)carbonyl]-oxy]propoxy]carbonyl]pentyl]pyridinium bromide (**9**) and 3-[6-[[2-methylene-3-[[(octadecylamino)carbonyl]oxy]-propoxy]carbonyl]hexyl]thiazolium bromide (**10**) were synthesized from 2-methylenepropane-1,3-diol (**5**). Platelet aggregation in platelet-rich plasma from rabbits, induced by racemic C_{16}-PAF, was competitively antagonized by **9** or **10**. At concentrations $\leq 10^{-4}$ M, neither compound **9** nor compound **10** caused platelet aggregation, nor did they inhibit platelet aggregation induced by collagen or adenosine diphosphate. Bronchoconstriction in the guinea pig and hypotension in the rat, induced by racemic C_{16}-PAF, were also effectively antagonized by **9** and **10**. Both appear to be more potent as PAF antagonists than Takeda's CV-3988.

Platelet activating factor (PAF) (**1**), an endogenous ether phospholipid identified as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkyl = hexadecyl, octadecyl),[1] exerts via receptor binding[2] a wide range of biological actions.[3] It induces platelet aggregation and smooth-muscle contraction, and in vivo experiments have demonstrated PAF's role in pathological processes,[3] such as asthma,[4] anaphylactic shock,[5] gastric ulceration,[6] and transplant rejection.[7]



1a: m = 15
1b: m = 17

A strong interest in the mode of action of PAF has led to the discovery of several structurally different PAF antagonists.[8] Most of the specific PAF receptor antagonists with PAF-like structures have retained a chiral carbon atom in the moiety that mimics the glycerol backbone of PAF. Such antagonists can be evaluated either as a racemate or as separate enantiomers. Generally, racemates are more readily synthesized than its separate enantiomeric components, but the pharmacological evaluation of a racemate may be a complicated and delicate matter.[9] In contrast to PAF and its unnatural enantiomer, which exhibit very different biological activities,[10] some chiral specific PAF antagonists with PAF-like structures demonstrate almost equal antagonist activity in either enantiomeric form.[11] Thus the chirality at the carbon atom

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