Tricyclic Compounds as Selective Muscarinic Receptor Antagonists. 3. Structure–Selectivity Relationships in a Series of Cardioselective (M_2) Antimuscarinics

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On the basis of the cardioselective muscarinic receptor antagonist AF-DX 116 (**2**), a series of 11-substituted pyridobenzodiazepinones (**9–35**) was prepared and screened for their binding affinity to muscarinic receptors located in cardiac (M_2) and glandular (M_3) tissue. The ratio of IC_{50} values of the test compounds in the two different tissues was taken as a measure of cardiac (M_2) receptor selectivity. Qualitative structure–selectivity relationships point to the fact that it is the spatial orientation of the protonated side-chain nitrogen atom in relation to the tricycle that is the main determinant for receptor subtype recognition and hence is important for the achievement of cardiac (M_2) selectivity.

The last decade has been characterized by a rapid growth of contributions directed toward the elucidation of the muscarinic system. This increasing interest resulted from evidence that the receptors of the muscarinic system consist of subpopulations that exhibit different structural, functional, and pharmacological properties.[1]

An important milestone along this line of research has been the discovery of the M_1 selective muscarinic antagonist pirenzepine (**1**), a compound that has been synthesized in our laboratories[2] and that is currently marketed as an antiulcer drug providing safe and unproblematic treatment of duodenal ulcers and gastritis.[3] The structure of pirenzepine is depicted in Figure 1. It consists of a butterfly-shaped tricyclic ring system and a (methylpiperazinyl)acetyl side chain, which both are connected by a carboxamide bond.

In recent years, pirenzepine has been extensively used as a pharmacological tool for the differentiation of muscarinic receptor subtypes. On the basis of the binding behavior of this compound, M_1 receptors have been defined as those muscarinic receptors that display a high affinity for pirenzepine. They are mainly located in neuronal and ganglionic tissue.[4]

M_2 receptors have been defined as binding sites with a low affinity for pirenzepine.[4] They are located in peripheral effector organs of the parasympathetic nervous system such as heart, smooth muscle, and glands and also in parts of the central nervous system. On the basis of this classification of M_1 and M_2 receptors, structure–selectivity relationships of M_1-selective antimuscarinics related to pirenzepine have been discussed in a preceding paper.[7]

However, evidence has accumulated in recent years that M_2 muscarinic receptors are not a homogeneous class of receptors but consist of different subtypes. One of the first compounds that strengthened this concept was the cardioselective muscarinic receptor antagonist AF-DX 116 (**2**), a substance that has been synthesized in our laboratories.[6] As can be seen (Figure 1), compound AF-DX 116 is closely related to the structure of pirenzepine. It contains the same tricyclic ring system, which is also connected to a basic side chain.

The side chain has been modified in such a way that the most basic nitrogen (pK_a 9.4) is not incorporated into the ring itself but is attached to it via a methylene bridge. This structural variation has led to a completely different selectivity profile as indicated in Figure 1.[8] AF-DX 116 exhibits in vitro a 10-fold higher affinity for receptors of the heart than for those of the cortex (M_1). In addition AF-DX 116 discriminates between muscarinic receptors of the heart and that of the submandibular or lacrimal gland by factors of more than 30. The selectivity of AF-DX 116 has been confirmed by in vivo binding studies and functional tests.[9,10] Thus, AF-DX 116 has become an important pharmacological tool providing evidence for heterogeneity of the M_2 class of muscarinic receptors. Unfortunately, to date there is no common, generally accepted nomenclature for the different muscarinic binding sites[11–14] in cardiac and glandular tissue. Following a proposal of Doods et al.,[5] we are classifying in this paper cardiac muscarinic receptors as M_2 and glandular receptors as M_3.

The surprising fact that minor structural variations in the side chain of the M_1 selective compound pirenzepine can lead to dramatic changes of the selectivity profile has prompted further work on the elucidation of cardiac M_2 selectivity. [Structural features that are decisive for M_1 or M_2 (cardiac) selectivity and a topographical characterization of the pharmacophores have been discussed by us at several scientific meetings.[15,16] The results of these studies will be published in one of our next papers.] Thus, a systematic variation of the structure of AF-DX 116 has been performed in order to identify those molecular fea-

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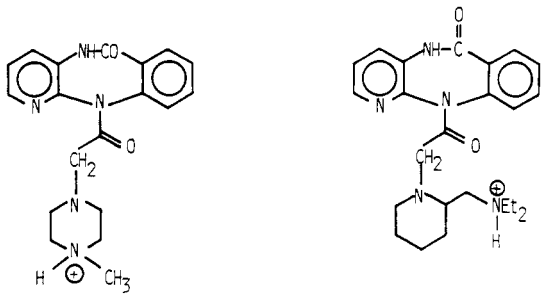


Pirenzepine (1)

M_1-Selective
Affinity profile:
$M_1 > M_3 \sim M_2$

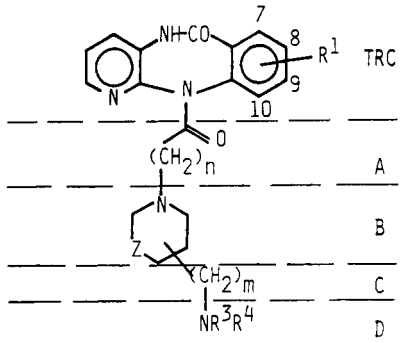
Antiulcer drug:
Treatment of duodenal ulcers and gastritis

AF-DX 116 (2)

M_2-Selective
Affinity profile:
$M_2 > M_1 >> M_3$

Experimental drug:
presumptive treatment of symptomatic bradycardia and sick sinus

Figure 1.



Structural variations of side chains and pyridobenzotricycle of AF-DX 116.

Z = Bond, CH_2, $(CH_2)_2$
n = 0, 1, 2
m = 1, 2
R^1 = H, 8-Cl, 9-Cl, 8-CH_3, 8-C_2H_5, 9-CH_3
NR^1R^2 as defined in Table I

Figure 2. Structural variations within the side chain and the pyridobenzo tricycle of AF-DX 116: Z = bond, CH_2, $(CH_2)_2$; n = 0, 1, 2; m = 1, 2; R^1 = H, 8-Cl, 8-CH_3, 8-C_2H_5, 9-Cl, 9-CH_3.

tures that are decisive for cardiac (M_2) selectivity (Figure 2). In the pursuit of this objective we focused our attention on compounds that discriminate between cardiac M_2 and glandular M_3 receptors. Preferential binding to the M_2 receptor versus M_3 receptor is important since inhibition of salivation is an unpleasant side effect of currently used nonselective antimuscarinic drugs.

Binding Studies

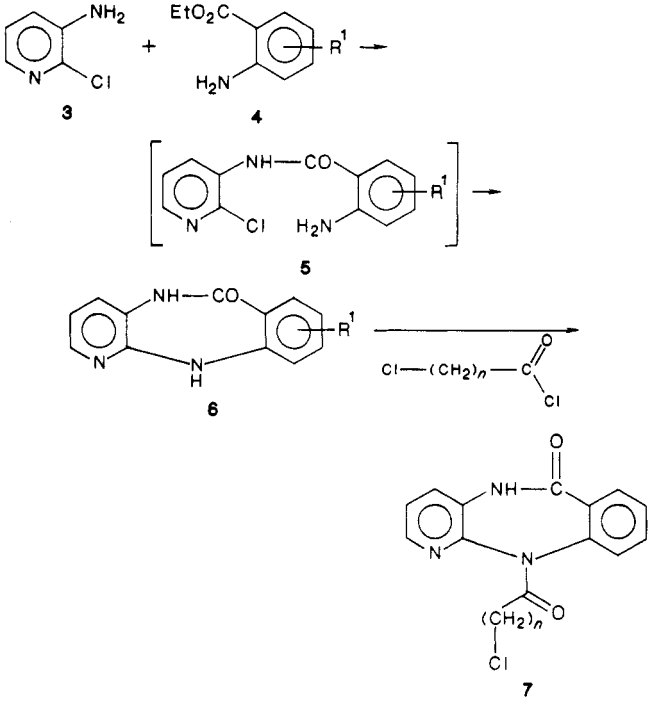
Binding studies were conducted in rat tissue homogenates. The nonselective radioligand [^{3}H]-*N*-methylscopolamine at a concentration of 0.3 nM was used to label muscarinic receptors in preparations of atria and submandibular gland containing prefenterially M_2 and M_3 receptors, respectively.

IC_{50} values (nM) for binding affinity to muscarinic receptors in the heart were taken as estimates for potency. The ratio of IC_{50} values of test compounds in the two different tissues, i.e. IC_{50} (gland)/IC_{50} (heart) = M_3/M_2, was taken as a measure of M_2 receptor selectivity.

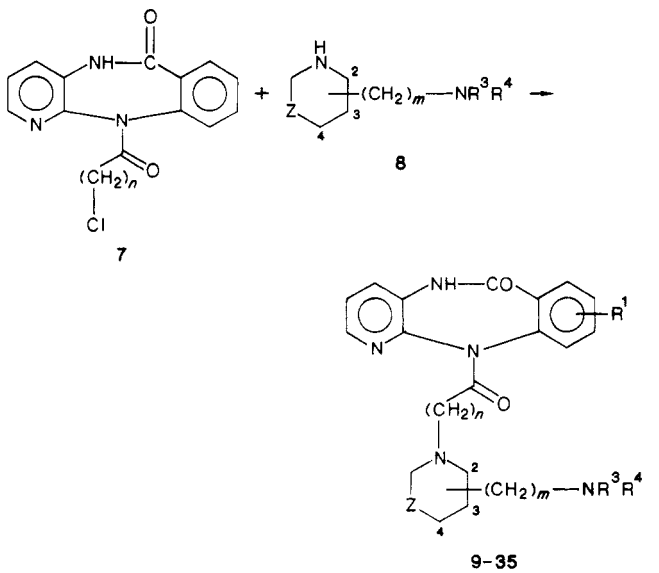
Chemistry

Table II lists the formulas and physical data of the new

Scheme I



Scheme II



compounds that were prepared according to known procedures.

The synthesis of the intermediate diazepinones (**6**) was readily accomplished by a one-pot synthesis as indicated in Scheme I. Condensation of 2-chloro-3-aminopyridine (**3**) with anthranilic esters (**4**) was effected in 1,2,4-trichlorobenzene in the presence of potassium *tert*-butoxide at 50 °C. The resulting anthranilic amides (**5**) were cyclized without further purification by addition of concentrated sulfuric acid to the reaction mixture and heating for 24 h at 115 °C and then another 2 h at 160 °C. The diazepinones (**6**) were obtained in yields between 50 and 70% (Table I).[17] Acylation of tricycles (**6**) with chloroacyl

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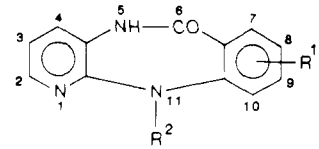
Table I. Physical Properties of Pyridobenzodiazepinones **6a–f** and **7a–f**



compd	R^2	R^1	yield, %	mp, °C	formula[a]	recrystn solvent
6a	H	9-Cl	60	>300	$C_{12}H_8ClN_3O$	DMF
6b	H	8-Cl	61	320–322	$C_{12}H_8ClN_3O$	DMF
6c	H	9-CH_3	53	286–288	$C_{13}H_{11}N_3O$	DMF
6d	H	8-CH_3	69	260–262	$C_{13}H_{11}N_3O$	1,2,4-trichlorobenzene
6e	H	8-Et	63	232–234	$C_{14}H_{13}N_3O$	70% acidic acid
6f	H	8-Br	60	310–311	$C_{12}H_8BrN_3O$	dimethylacetamide
7a	$COCH_2Cl$	9-Cl	90	226–230	$C_{14}H_9Cl_2N_3O_2$	DMF
7b	$COCH_2Cl$	8-Cl	77	211–212	$C_{14}H_9Cl_2N_3O_2$	ethanol
7c	$COCH_2Cl$	9-CH_3	93	205–206	$C_{15}H_{12}ClN_3O_2$	dioxane
7d	$COCH_2Cl$	8-CH_3	64	228–230	$C_{15}H_{12}ClN_3O_2$	ethylene glycol
7e	$COCH_2Cl$	8-Et	81	200–201	$C_{16}H_4ClN_3O_2$	acetonitrile
7f	$COCH_2Cl$	8-Br	37	222–223	$C_{14}H_9BrClN_3O_2$	dioxane

halides furnished the corresponding ω-chloroacyl amides in good yield. The base-substituted condensed diazepinones **9–35** were obtained by reaction of the haloacyl compounds **7** with secondary amines of type **8** as depicted in Scheme II. The secondary amines of type **8** have been prepared by known procedures as indicated in the Experimental Section. AF-DX 116 contains an asymmetric carbon atom in the side chain. The preparation of the individual enantiomers **28** and **29** has been achieved by reaction with the respective chiral side chain amines as described in Scheme II.

Biological Results and Discussion

The derivatives studied are listed in Table II along with their binding affinities to the cardiac (M_2) and glandular muscarinic (M_3) receptors.

In preceding papers the requirements for the achievement of M respective M_1 selectivity have been discussed.[7,18] This analysis suggests that M_1 selectivity of a series of pirenzepine analogues arises mainly from conformational effects. Assuming that these compounds bind to the M receptor via a three-point attachment, it can be envisioned that the three structural features defining the M_1 selective pharmacophore are the two aromatic rings and the strongly basic nitrogen atom of the side chain that they all have in common. Moreover, if we assume that the aromatic rings of the tricycle are accommodated at the receptor in a very similar fashion, it is very likely that the placement of the protonated nitrogen determines receptor selectivity. This view gains support from a simple comparison of the structures of pirenzepine and AF-DX 116. Both compounds contain the same tricyclic ring system and yet exhibit different receptor selectivity, a situation that clearly reflects the important influence of the side chain for receptor selectivity.

In order to gain a deeper insight into the question whether M_3/M_2 selectivity of AF-DX 116 and its analogues is determined by similar stereochemical requirements, we dissected the molecule of AF-DX 116 into five different segments (Figure 2) and studied the influence of the individual structural elements on binding affinity and M_3/M_2 selectivity.

Tricycle. In view of the anticipated minor influence of the tricycle, variations of this moiety have been restricted to some minor changes, i.e. introduction of small substituents in the benzene ring of the pyridobenzodiazepinone system. Representatives of this type are compounds **30–35**, which bear halogen or alkyl substituents in positions 8 or 9. All compounds are cardioselective antagonists with M_3/M_2 ratios between 10 and 40. A slight increase in binding affinity is achieved by the introduction of small lipophilic substituents such as chlorine or methyl.

Segment A. A comparison of compounds **9**, **10**, and **11** demonstrates the influence of the alkyl chain length *n* in segment A. Compound **11** is nonselective. With *n* = 0 (**9**), the binding affinity is dramatically reduced.

Segment B. The influence of the position of the (dialkylamino)alkyl substituent in the piperidine ring is demonstrated by a comparison of compounds **10**, **14**, and **15**. A shift from position 2 to 3 or 4 at the piperidine ring leads to significant reduction in selectivity and in the case of compound **15** to a marked decrease in binding affinity as well.

Exchange of the piperidine ring in AF-DX 116 (**10**) by the corresponding pyrrolidine (Z = bond, Figure 2) or hexahydro-1*H*-azepine (Z = CH_2CH_2) ring (compounds **16** and **17**) leads to compounds with reduced selectivity and in the case of compound **17** (Z = CH_2CH_2) to a significant loss of affinity.

Segment C. Quite similar relations were found for the influence of the alkyl chain length *m* in segment C. It is evident from a comparison of compounds **10**, **12**, and **13** that the number of CH_2 increments is a crucial determinant of selectivity whereas the binding properties remain unaffected; i.e. prolongation of the chain length (*n* = 2 and 3) leads to nonselective compounds.

Segment D. Compounds with a broad variety of substituents at the most basic N atom are represented by substances **18–27**. Evidently the type of alkyl substituent attached to the N atom exerts a strong influence on M_3/M_2 selectivity. Even an exchange of a diethylamino by a dimethylamino group leads already to a marked reduction of selectivity. The binding affinity however remains unaffected.

Enantioselectivity. As expected, the enantiomeric forms of AF-DX 116 (compounds **28** and **29**) exhibit different binding characteristics for the muscarinic receptor. The dextrorotatory enantiomer **29** discriminates between muscarinic receptors of the heart and the gland by more than a factor of 100 and constitutes thus in this series of compounds the absolute lead.

Summarizing the results obtained above as qualitative structure–activity and structure–selectivity relationships, we can draw the following conclusions.

(17) German Patent 3222809 (18.06.1982).

M_2 selective compounds are characterized by flexible side chains. Only compounds in close structural relationship to AF-DX 116 exhibit a reasonable degree of selectivity for cardiac muscarinic receptors. All structural variations with respect to the chain length in segments A and C, position of the amino alkyl group attached to the piperidinyl ring of segment B, and size of substituents at the amino nitrogen atom in segment D give rise to a dramatic decrease in M_3/M_2 selectivity.

Even though the interactive influences of the individual elements of the side chain are not completely clear, the above results might be taken as a support for our view that M_2 selectivity is mainly controlled by conformational effects. We think that it is the spatial orientation of the protonated nitrogen atom in relation to the tricycle that is of major importance for M_3/M_2 selectivity. A deeper insight into the conformational requirements for the achievement of M_3/M_2 selectivity can be gained only by a rigorous conformation space analysis. Further work in this direction is currently under way.

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.

Halogen- and alkyl-substituted anthranilic acids of type **4** used as starting material for the synthesis of pyridodiazepinones **6** are commercially available.

The secondary amines **8**, e.g. 2-,[19-21] 3-,[22,23] or 4-[(dialkylamino)alkyl]piperidines,[24] 2-[(dialkylamino)methyl]pyrrolidines,[25] and 2-[(dialkylamino)methyl]hexahydro-1*H*-azepines[26] were prepared as described in the literature.

General Procedure for the Preparation of Pyridobenzodiazepinones 6a–f. Potassium *tert*-butoxide (58.4 g, 0.52 mol) was added to a suspension of 2-chloro-3-aminopyridine (51.4 g, 0.4 mol) in 1,2,4-trichlorobenzene (160 mL). Methyl 2-amino-5-methylbenzoate (85.9 g, 0.52 mol) was added within 30 min, and the mixture was stirred for 1 h at 50 °C. After dilution of the reaction mixture with 1,2,4-trichlorobenzene (220 mL), concentrated sulfuric (22.8 mL) was added and the *tert*-butyl alcohol distilled off in vacuo. The reaction mixture was stirred for 24 h at 115 °C and then for another 2 h at 160 °C. After completion of the reaction, the mixture was cooled to room temperature, and the suspension of crystals was collected by filtration and washed with petroleum ether. The filter cake was suspended in 50% aqueous acetone (670 mL), concentrated ammonia (60 mL) was added, and the mixture stirred for 1 h at 50 °C. The crystal slurry was collected by filtration, washed with water, and dried at 50 °C. Colorless crystals: mp 260–262 °C; yield 62.55 g (69.5%); ^{1}H NMR (200 MHz, DMSO) δ 9.82 (s, 1 H), 8.36 (s, 1 H), 7.87 (dd, 1 H), 7.51 (d, 1 H), 7.28 (dd, 1 H), 7.18 (dd, 1 H), 7.02 (d, 1 H), 6.92 (dd, 1 H), 2.2 (s, 3 H); IR (KBr) 3260, 3190, 1675, 1605 cm^{-1}. Anal. ($C_{13}H_{11}N_3O$) C, H, N.

Compounds **6a,b,c,e,f** were prepared by the same procedure. The isolated compounds were dried and recrystallized from solvents as indicated in Table I.

General Procedure for the Preparation of 11-(Chloroacetyl)pyridobenzodiazepinones 7a–f. 5,11-Dihydro-8-methyl-11-(chloroacetyl)-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (7d). Chloroacetyl chloride (4.31 mL, 0.054 mol) and triethylamine (7.5 mL) were added simultaneously within 30 min to a solution of **6d** (10.0 g, 0.045 mol) in dioxane (100 mL) at 100 °C. The reaction mixture was stirred at reflux for another 5 h. After cooling, the precipitate was removed by filtration, and the solvents were evaporated in vacuo to afford a white solid. Recrystallization from ethylene glycol afforded 8.6 g (64%) of **7d**: mp 228–230 °C; ^{1}H NMR (200 MHz, DMSO) δ 10.8 (s, 1 H), 8.29 (dd, 1 H), 7.7 (dd, 1 H), 7.62 (s, 1 H), 7.47 (m, 2 H), 7.40 (m, 1 H), 4.33 (s, 2 H), 2.35 (s, 3 H); IR (KBr) 3200, 1705, 1660 cm^{-1}. Anal. ($C_{15}H_{12}ClN_3O_2$) C, H, N.

Compounds **7a,b,c,e,f** were prepared according to the same method. The isolated compounds were recrystallized from solvents as indicated in Table I.

General Procedure for the Preparation of Pyridobenzodiazepinones 9–35. 11-[[2-[(Diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**10**). A mixture of 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (64.0 g, 0.393 mol), 2-[(diethylamino)methyl]piperidine (36.0 g, 0.34 mol), sodium carbonate (37.9 g, 0.36 mol), and acetonitrile (780 mL) was refluxed for 5 h with stirring. After cooling, the mixture was filtered and the residue rinsed twice with acetonitrile (50 mL). Recrystallization from 1-propanol afforded colorless crystals, mp 228–230 °C, in a yield of 88.2 g (62%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.65 (br, 1 H), 8.3 (s, 1 H), 7.95 (d, 1 H), 7.65 (m, 3 H), 7.42 (s, 1 H), 7.30 (m, 1 H), 4.2 (m, 0.5 H), 3.7 (s, 1 H), 3.17 (m, 0.5 H), 2.78 (m, 0.5 H), 2.55 (s, 1 H), 2.40 (m, 4.5 H), 2.10 (m, 2 H), 2.7–1.1 (m, 6 H), 0.9 (m, 6 H); IR (KBr) 3210, 1680, 1665, 1655 cm^{-1}. Anal. ($C_{24}H_{31}N_5O_2$) C, H, N.

Compounds **9** and **11–35** were prepared in an analogous manner; melting points and yields are given in Table II. In some cases it was necessary to use column chromatography with silica gel for purification using acetonitrile/dichloromethane/ethyl acetate/cyclohexane/methanol/concentrated ammonia (6:3.5:1.5:0.46:0.46:0.06) as eluant.

(+)-11-[[2-[(Diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one. (a) (−)-2-[(Diethylamino)methyl]piperidine. Solutions of racemic 2-[(diethylamino)methyl]piperidine (45.2 g, 0.625 mol) in methanol (132 mL) and L-(+)-tartaric acid (93.0 g, 0.62 mol) in methanol (264 mL) were mixed, and the mixture was left to stand overnight at ambient temperature. The resulting precipitate was collected by filtration, washed with methanol, and decanted with methanol (250 mL) for 30 min. The mixture was recrystallized from ethanol/water (4:1 v/v) four times, and colorless crystals (29.9 g) were obtained, mp 191–192.5 °C, which were identified as the ditartrate. Anal. Calcd for $C_{10}H_{22}N_2 \cdot 2C_4H_6O_6$: C, 45.95; H, 7.28; N, 5.95. Found: C, 46.06; H, 7.08; N, 5.96.

The product was treated with potassium hydroxide solution, and after the usual workup, the desired free base was obtained: bp (17 mmHg) 88–94 °C; $[\alpha]^{20}_D$ −68° (ethanol).

In order to determine the enantiomeric purity, a sample of the base was converted with (*S*)-(−)-1-phenylethyl isocyanate into the corresponding urea and subsequently investigated by HPLC. The content in the base of (−)-enantiomer according to this procedure was at least 98.9%.

(b) (+)-11-[[2-[(Diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (29). The title compound was prepared according to the general procedure for the preparation of pyridobenzodiazepinones **9–35** from 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-benzodiazepin-6-one and (−)-2-[(diethylamino)methyl]piperidine to give colorless crystals: mp 210–211.5 °C (1-propanol; $[\alpha]^{20}_D$ +11.4° (dilute aqueous hydrochloride acid). The dihydrobromide melts at 241–242 °C (with decomposition; from ethanol). Anal. ($C_{24}H_{31}N_5O_2$) C, H, N.

(−)-11-[[2-[(Diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one. (a) (+)-2-[(Diethylamino)methyl]piperidine. The title

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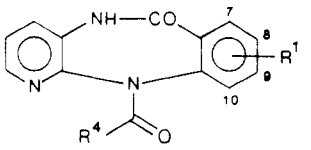
Table II. Physical Properties of Substituted Tricyclic Pyridobenzodiazepinones and Their Binding Affinities to Cardiac and Glandular Muscarinic Receptors



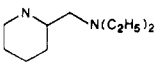
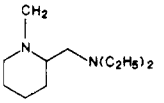
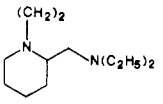
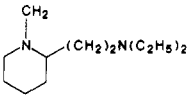
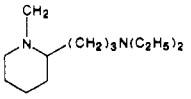
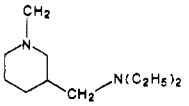
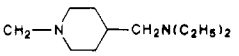
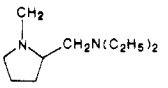
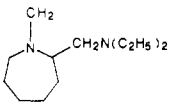
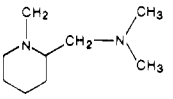
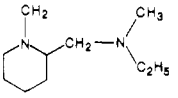
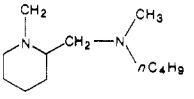
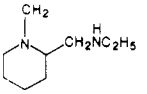
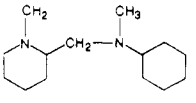
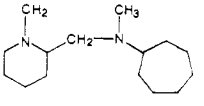
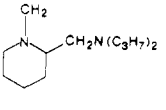
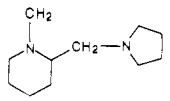
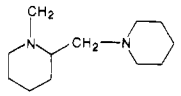
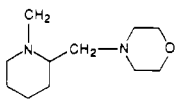
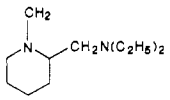
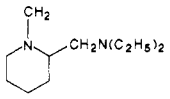
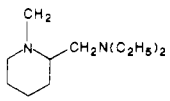
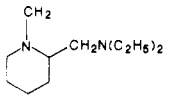
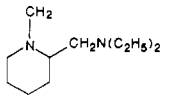
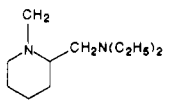
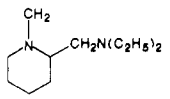
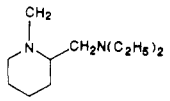
no.	R^1	R^4	recrystn solvent	mp, °C	yield,[a] %	mol[b] formula	IC_{50}, nM		
							M_2	M_3	M_3/M_2[d]
9	H			amorphous	23	$C_{23}H_{29}N_5O_2$	5000	>10000	>2
10	H		1-propanol	228–230	62	$C_{24}H_{31}N_5O_2$	140	6000	42.8
11	H		acetonitrile	160–162	56	$C_{25}H_{33}N_5O_2$	220	600	2.7
12	H		acetonitrile	137–139	41	$C_{25}H_{35}N_5O_2$	330	1000	3.0
13	H		acetonitrile	151–153	74	$C_{26}H_{35}N_5O_2$	60	200	3.3
14	H		ethyl acetate	199–200	59	$C_{24}H_{31}N_5O_2$	170	300	1.8
15	H		diethyl ether	163	58	$C_{24}H_{31}N_5O_2$	800	4000	5
16	H		ethyl acetate	212 dec	13	$C_{23}H_{29}N_5O_2$	150	2000	13
17	H		ethyl acetate/methanol	139 dec	45	$C_{25}H_{33}N_5O_2$	2000	15000	7.5
18	H		acetonitrile	189–190	43	$C_{22}H_{27}N_5O_2$	140	1000	7.1
19	H		acetonitrile	200–202	24	$C_{23}H_{29}N_5O_2$	120	1500	12.5
20	H		acetonitrile	155–156	35	$C_{25}H_{33}N_5O_2$	100	200	2.0
21	H		acetonitrile	182–186	52	$C_{22}H_{27}N_5O_2 \cdot 2HCl$	600	3000	5
22	H		acetonitrile	175–177	65	$C_{27}H_{35}N_5O_2$	60	150	2.5
23	H		acetonitrile	184–185	80	$C_{28}H_{37}N_5O_2$	30	70	2.3
24	H		acetonitrile	154–156	76	$C_{26}H_{35}N_5O_2$	1500	6000	4.0

Table II (Continued)

no.	R^1	R^4	recrystn solvent	mp, °C	yield,[a] %	mol[b] formula	IC_{50}, nM M_2	M_3	M_3/M_2[d]
25	H		acetonitrile	230–231	25	$C_{24}H_{29}N_5O_2$	560	1500	2.7
26	H		ethanol	212–214	50	$C_{25}H_{31}N_5O_2$	600	1500	2.5
27	H		acetonitrile	203–205	54	$C_{24}H_{29}N_5O_3$	>1000	4000	<4
28	H		1-propanol	210–211.5	53	$C_{24}H_{31}N_5O_2$	400	10000	25
29	H		1-propanol	210–211.5	51	$C_{24}H_{31}N_5O_2$	50	6000	120
30	9-Cl		acetonitrile	167.5–169	35	$C_{24}H_{30}ClN_5O_2$	40	1500	37.5
31	8-Cl		acetonitrile	186–188	48	$C_{24}H_{30}ClN_5O_2$	100	4000	40.0
32	9-CH_3		acetonitrile	172–174	50	$C_{25}H_{33}N_5O_2$	70	2000	28.5
33	8-CH_3		diisopropyl ether	173–174	54	$C_{25}H_{33}N_5O_2$	30	1000	33.3
34	8-C_2H_5		diisopropyl ether	141–143	50	$C_{26}H_{35}N_5O_2$	200	2000	10
35	8-Br		acetonitrile	165–167	64	$C_{26}H_{30}BrN_5O_2$	150	4000	26.6

[a] No attempt was made to optimize yields. Numbers represent the overall yield for the last step. [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] Pirenzepine has been used as reference compound with the following IC_{50} (nM) values: heart, 800; gland, 600; M3/M2 ~ 1.

compound was prepared analogously from racemic 2-[(dimethylamino)methyl]piperidine and D-(−)-tartaric acid. The ditartrate melted at 191–192.5 °C. The base, with a boiling point of 88–94 °C at 17 mmHg, had a content of (+)-enantiomer of 98.5%, determined by reaction with (*S*)-(−)-phenylethyl isocyanate and subsequent HPLC investigation; $[\alpha]^{20}_D$ +64° (ethanol).

(b) (−)-11-[[2-[(Diethylamino)methyl]-1-piperidinyl]-acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (28). The title compound was prepared analogously to **29** from 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one and (+)-2-[(diethylamino)methyl]piperidine to give colorless crystals: mp 210–211.5 °C (1-propanol/activated charcoal); $[\alpha]^{20}_D$ −12° (dilute aqueous hydrochloride acid). Anal. ($C_{24}H_{31}N_5O_2$) C, H, N.

Biochemistry. Receptor-Binding Assay. Radioligand Binding Studies. Rats were killed by cervical dislocation. Tissues were removed, cleaned of adhering matter, and homogenized (submandibular gland, 1:150; total heart, 1:300) with an Ultra-Turrax at maximal speed for 30 s and then with a Potter-Evelhjem (15 strokes) in Na^+/Mg^{2+} HEPES buffer, pH 7.4 (100 mM NaCl, 10 mM $MgCl_2$, 20 mM HEPES) and filtered through two layers of cheesecloth. Binding curves for the different compounds were derived indirectly from competition experiments against 0.3 nM [^{3}H]NMS for both tissues. One milliliter of homogenate was incubated for 45 min at 30 °C in the presence of the marker ligand and different concentrations of the cold ligand were added, conditions under which equilibrium was reached as determined by appropriate association experiments (unpublished data). The incubation was terminated by centrifugation (12 000 rpm for 3 min) at room temperature with an Eppendorf microcentrifuge. The resultant pellet was washed twice with 1.5 mL of saline to remove the free radioactivity and the final pellet was allowed to drain. The tips of the tubes containing the pellet were cut off and 200 μL of tissue solubilizer (Lumasolve, Lumac) were added and left to stand overnight. Radioactivity was then counted after addition of 4 mL of liquid scintillation mixture (dimilume/toluene 1:10 v:v, Packard). 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 1 μM 3-quinuclidinyl benzilate racemic mixture (QNB) in [^{3}H]NMS experiments. Nonspecific binding averaged less than 30%.

Acknowledgment. We thank Hubertus Jahn and Wolfgang Uhl (Department of Chemical Research) for their excellent technical assistance in the synthetic work.

Registry No. **3**, 6298-19-7; **4** (R^1 = 4-Cl), 5900-58-3; **4** (R^1 = 5-Cl), 5202-89-1; **4** (R^1 = 4-CH_3), 18595-17-0; **4** (R^1 = 5-CH_3), 18595-16-9; **4** (R^1 = 5-Et), 2475-78-7; **4** (R^1 = 5-Br), 52727-57-8; **6a**, 1022-30-6; **6b**, 114368-11-5; **6c**, 118306-19-7; **6d**, 1214-92-2; **6e**, 118306-15-3; **6f**, 118306-17-5; **7a**, 28797-51-5; **7b**, 28797-50-4; **7c**, 120990-79-6; **7d**, 28797-53-7; **7e**, 120990-80-9; **7f**, 120990-81-0; **7** (R^1 = H, n = 0), 87571-90-2; **7** (R^1 = H, n = 1), 28797-48-0; **7** (R^1 = H, n = 2), 31265-81-3; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 100158-61-0; (±)-**8** (Z = CH_2, m = 2, R^3 = R^4 = C_2H_5, 2-position), 120990-82-1; (±)-**8** (Z = CH_2, m = 3, R^3 = R^4 = C_2H_5, 2-position), 120990-83-2; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 3-position), 120990-84-3; **8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 4-position), 116905-90-9; (±)-**8** (Z = bond, m = 1, R^3 = R^4 = C_2H_5, 2-position), 121053-95-0; (±)-**8** (Z = $(CH_2)_2$, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-85-4; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = CH_3, 2-position), 100158-60-9; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = C_2H_5, 2-position), 120990-86-5; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = C_4H_9, 2-position), 120990-87-6; (±)-**8** (Z = CH_2, m = 1, R^3 = C_2H_5, R^4 = H, 2-position), 120990-88-7; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = C_6H_{11}, 2-position), 121053-96-1; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = cycloheptyl, 2-position), 120990-89-8; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_3H_7, 2-position), 120990-90-1; (±)-**8** (Z = CH_2, m = 1, NR^3R^4 = pyrrolidino, 2-position), 112282-40-3; (±)-**8** (Z = CH_2, m = 1, NR^3R^4 = piperidino, 2-position), 120990-91-2; (±)-**8** (Z = CH_2, m = 1, NR^3R^4 = morpholino, 2-position), 120990-92-3; (−)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-93-4; (−)-**8**·2L-tartrate (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-95-6; (+)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-94-5; (+)-**8**·2D-tartrate (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-96-7; **9**, 120990-62-7; **10**, 100158-38-1; **11**, 100158-14-3; **12**, 121011-73-2; **13**, 120990-63-8; **14**, 100158-44-9; **15**, 120990-64-9; **16**, 121054-51-1; **17**, 120990-65-0; **18**, 100158-13-2; **19**, 121011-74-3; **20**, 120990-66-1; **21**, 120990-67-2; **22**, 120990-68-3; **23**, 120990-69-4; **24**, 120990-70-7; **25**, 120990-71-8; **26**, 120990-72-9; **27**, 120990-73-0; (−)-**28**, 100158-38-1; (+) **29**, 121029-35-4; (+) **29**·2HBr, 120990-97-8; **30**, 114367-96-3; **31**, 120990-74-1; **32**, 120990-75-2; **33**, 120990-76-3; **34**, 120990-77-4; **35**, 120990-78-5; $ClCH_2COCl$, 79-04-9.

Synthesis of Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones as Potential Antileukemic Agents

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The synthesis of unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones bearing one "mitoxantrone side arm" and another (aminoalkyl)amino moiety has been described. These unsymmetrical anthracene-9,10-diones exhibit cytotoxic activity against L1210 leukemia cells and antitumor activity against P388 leukemia in mice.

Ametantrone, 1,4-bis[[2-[(2-hydroxyethyl)amino]-ethyl]amino]anthracene-9,10-dione (**1**) and its 5,8-dihydroxy analogue, mitoxantrone (**2**), have shown outstanding antineoplastic activity and are among the most promising anticancer agents.[1,2] These compounds are thought to exert their cytotoxic effect by an intercalation into DNA mechanism.[3,4] Properly designed amino-substituted side chains may stabilize the intercalated planar chromophore by interacting with the sugar and phosphate units of DNA and modify its conformation and function. Therefore, a number of analogues of **1** and **2** with various (aminoalkyl)amino side chains localized in different positions of the 9,10-anthracenedione ring were synthesized for structure–activity relationship evaluation.[2,5-9] One of the studied groups of anthracenediones are compounds with nonidentical side chains. It has been shown that the simplest compounds of that type, with one "mitoxantrone arm" at position 1 and a hydroxyl or amino group at position 4, exhibit considerable antitumor activity.[6,9] In another synthetic approach unsymmetrically 1,4-bis-substituted anthracenediones were obtained by a two step photolytic–thermolytic procedure, starting from 1,4-dimethoxyanthracene-9,10-dione.[10] However, the method applied allowed the synthesis of compounds within a limited range of structures of side chains. None of the obtained compounds exhibited any significant antileukemic activity although some of them were active in vitro.[11] Several unsymmetrical 1,4-diaminoanthraquinones have been also synthesized by Zielske,[12] but antineoplastic activity of these derivatives has not been evaluated.

In this report we describe the synthesis of a number of unsymmetrically 1,4-bis-substituted anthracene-9,10-diones with a "mitoxantrone side arm" at position 1 and with different alkylamino chains at position 4 (Figure 1; **5**, **6**, **8**, **10**, and **12**). These compounds contain the basic

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(1) Wallace, R. E.; Murdock, K. C.; Angier, R. B.; Durr, F. E. *Cancer Res.* **1979**, *39*, 1570.
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