Synthesis of New Cardioselective M_2 Muscarinic Receptor Antagonists

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A series of 5*H*-dibenz[*b*,*f*]azepine derivatives was prepared and evaluated for binding affinities to muscarinic receptors *in vitro*. Among them, compound 8 showed a high affinity for human recombinant M_2 receptors (K_i=2.6 nM), a low affinity for M_4 receptors (39-fold less than for M_2 receptors) and a very low affinity for M_1 and M_3 receptors (119- and 112-fold less than for M_2 receptors, respectively). The high M_2 selectivity of 8 may be attributed to the olefinic bond of the azepine ring. Functional experiments showed 8 to be a competitive antagonist with high affinity to the cardiac (pA_2=7.1) and low affinity to the intestinal muscarinic receptors (IC_{50}=0.54 μM). *In vivo* experiments confirmed the *in vitro* M_2 selectivity of 8. Acetylcholine-induced bradycardia was dose-dependently antagonized in rats after both intravenous and intraduodenal administration of 8. In rats, cholinergic functions mediated by M_1 or M_3 receptors (salivary secretion, pupil diameter, gastric emptying, intestinal transit time) were not affected by the oral administration of 8 even at doses as high as 30 times the antibradycardic effective dose. Furthermore, 8 had no analgesic activity in mice, indicating poor central nervous system penetration. In dogs, nocturnal bradycardia was dose-dependently inhibited by the oral route with a duration of action of about 24 h. Compound 8 appears to be a promising cardioselective antimuscarinic agent for the treatment of dysfunctions of the cardiac conduction system such as sinus or nodal bradycardia ("sick-sinus syndrome") and atrioventricular block.

Key words 5*H*-dibenz[*b*,*f*]azepine derivative; M_2 muscarinic receptor; muscarinic antagonist; M_2 selectivity; bradycardia

Acetylcholine acts through receptors located on the cell membrane surface. These receptors are classified as nicotinic or muscarinic receptors on the basis of their response to alkaloid nicotine and muscarine. Muscarinic receptors modulate a number of important functions including nerve-to-nerve transmission, smooth-muscle contraction, and exocrine and endocrine secretion. They play a major role in many pathologic conditions.[1)]

Four muscarinic receptor subtypes have been characterized pharmacologically (M_1, M_2, M_3 and M_4), while five molecular forms have been identified and cloned (m_1, m_2, m_3, m_4 and m_5).[2)] Muscarinic receptors are coupled to G-proteins, which in turn modulate the activity of a different second messenger (phospholipase C and adenylyl cyclase).[3)] The use of muscarinic agonists or antagonists has been limited by their low specificity. Due to the fact that muscarinic receptors are found in many tissues, a therapeutic effect in one tissue is often accompanied by unwanted effects in another.[4)]

The cardiac conduction system and cardiac muscle have postsynaptic M_2 receptors that mediate bradycardia and decrease cardiac contractility.[5)] Cortex and hippocampus have presynaptic M_2 receptors (autoreceptors) which, when stimulated, inhibit the release of acetylcholine.[6)] The loss of presynaptic muscarinic receptors has been implicated in the etiology of Alzheimer's disease.[7)] In the heart, the overactivation of M_2 muscarinic receptors *via* the vagus nerve is believed to play a major role in the etiology of the autonomic (neuromediated) sick sinus syndrome.[8,9)] Thus, M_2 muscarinic receptor antagonists may be useful in the treatment of sinus node dysfunction caused by vagus hypertone, and may postpone or replace permanent endocardial pacing for patients with contraindications.[10)] Atropine, a non-selective muscarinic receptor antagonist, was shown to be effective in increasing the heart rate of patients with sick sinus syndrome.[11)] However, its use is limited by the short duration of action and the occurrence of unwanted side effects such as dry mouth, mydriasis, constipation and urinary retention caused by the antagonism of other subtypes.

Different groups have reported the synthesis of selective M_2 antagonists: Boehringer Ingelheim with AF-DX 116 (**1**) and AQ-RA 741 (**2**) derivatives (Fig. 1),[12,13)] Yamanouchi with YM-47244 and YM-55758 derivatives,[14—17)] George Washington University with DIBA (**3**) and DIBD derivatives,[18,19)] University of Bologna with methoctramine and tripitramine derivatives,[20—23)] University of Bonn with W84 and WDUO derivatives[24)] and Schering–Plough with SCH 57790 derivatives.[25)] However, most of the reports do not provide information on the selectivity of M_2 antagonists *versus* M_4 receptors which has recently been recognized to play an important role in respiratory physiology.[26)] In addition, many reports used putative receptor subtypes from tissue or cell preparations that do not express a single receptor subtype. We have synthesized a new group of selective M_2 muscarinic receptor antagonists (**4**) by modification of DIBA (**3**), as depicted in Fig. 2. We replaced the amide bond of the tricyclic ring system of DIBA with the bioisoster olefinic bond[27—29)] differently substituted. In addition, we have explored the effect of increased flexibility of the side chain of DIBA by cleavage of the piperidine ring.[14)] We have also determined the optimal distances between the carbonyl carbon and the two amine groups (Fig. 2).

Receptor binding affinities of the compounds were evaluated using human recombinant muscarinic receptors subtypes M_1, M_2, M_3 and M_4.

Chemistry

5*H*-Dibenz[*b*,*f*]azepine derivatives **8**—**17** and **19**—**24** (Tables 1 and 2) were prepared according to Chart 1. The key

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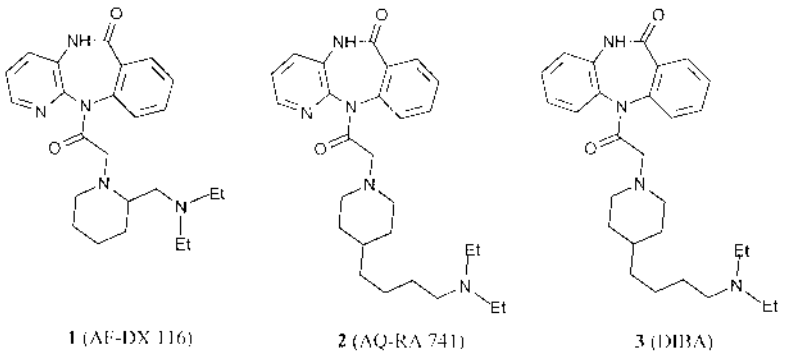


Fig. 1. Chemical Structure of AF-DX 116, AQ-RA 741 and DIBA

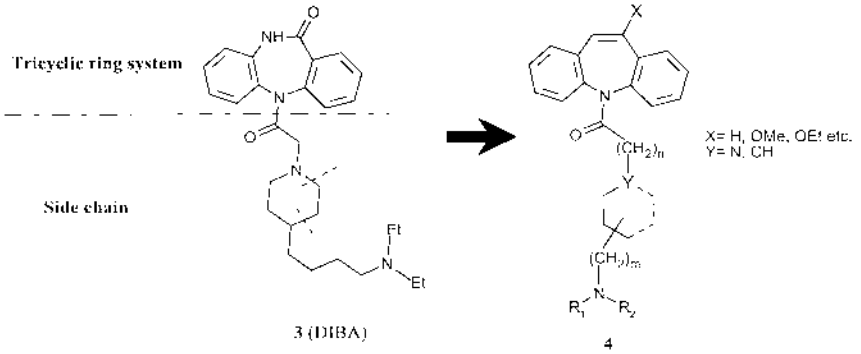


Fig. 2. Structural Variations within the Tricyclic Ring and the Side Chain of DIBA

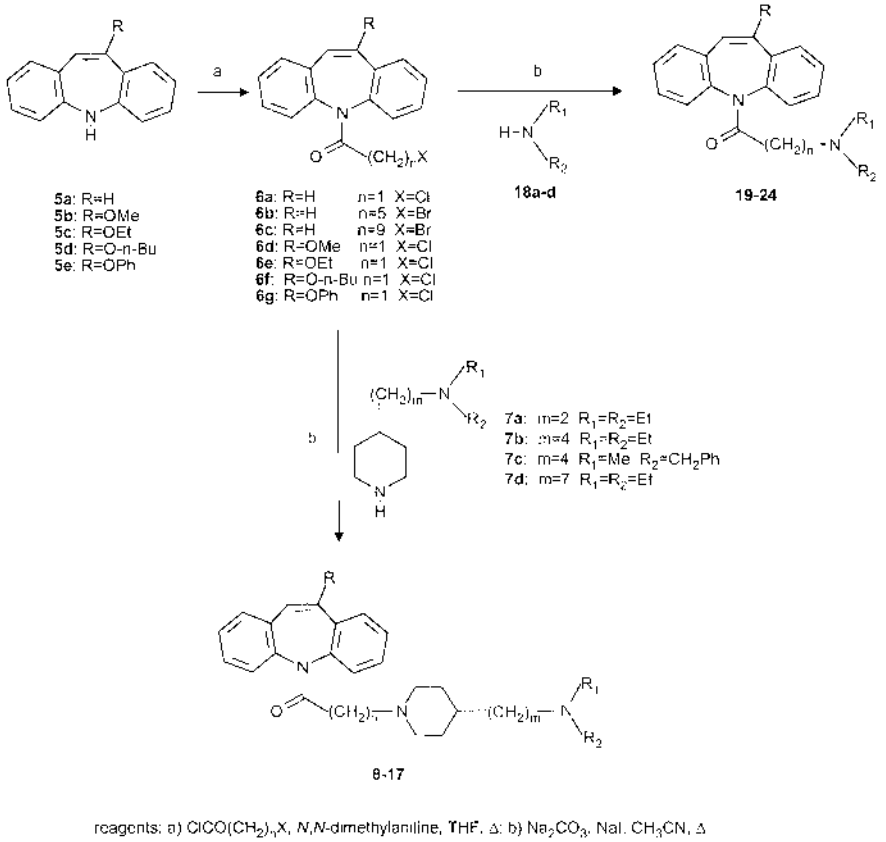


Chart 1. Preparation of the 5*H*-Dibenz[*b*,*f*]azepine Derivatives **8**—**17** and **19**—**24**

intermediates **6a**—**g** were prepared from the corresponding ϖ-haloacyl chlorides and the appropriate dibenz[*b*,*f*]-azepines **5**. Reaction of **6a**—**g** with different amine compounds, **7a**—**d** and **18a**—**d**, in acetonitrile in the presence of sodium carbonate afforded target compounds. Alkoxy-dibenz[*b*,*f*]azepine **5b**—**d** were prepared according to the

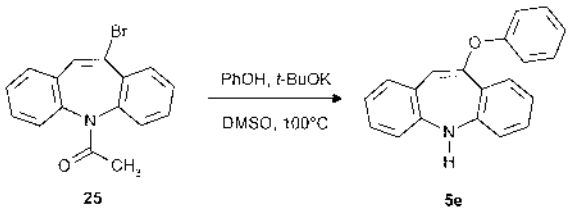
method reported by Haász,[30] while the 5-phenoxy-dibenz[*b*,*f*]azepine **5e** was synthesized starting from 5-acetyl-10-bromo-5*H*-dibenz[*b*,*f*]azepine **25**,[31] as shown in Chart 2. Treatment of compound **25** with *t*-BuOK in the presence of phenol yields **5e**, probably *via* a hetaryne intermediate.[32]

Compounds **8**—**17** were obtained by the corresponding **6a**—**g** and 4-(dialkylaminoalkyl)piperidines **7a**—**d**.[33]

Chart 3 shows the preparation of **7b**, **c** starting from *N*-benzyl-piperidone **26**. **26** was treated with triethyl phosphonocrotonate (Horner–Emmons reaction) to afford **27**, which was easily hydrogenated and benzylated to give **28b**. Conversion of ethylester **28b** to alcohol **29a**, followed by reaction with methanesufonyl chloride, gave **29b**. Treatment of compound **29b** with diethylamine, followed by debenzylation, gave piperidine derivative **7b**. Compound **29b** was also used to prepare compound **7c**. **29b** was treated with methylamine and acylated with benzoyl chloride to afford **32a**, which was easily reduced and debenzylated to give piperidine derivative **7c**.

The synthetic route for preparation of the piperidine derivative **7d** is illustrated in Chart 4. Pyridine derivative **35** was obtained by the alkylation of 4-picolylsodium with bromoderivative **34**. Catalytic reduction of compound **35** over platinum oxide, followed by reduction with lithium aluminium hydride, gave **7d**.

Chart 5 reports the synthesis of a corresponding hydrogenated analog of compound **8**, starting from iminodibenzyl derivative **37** and amine **7b**.



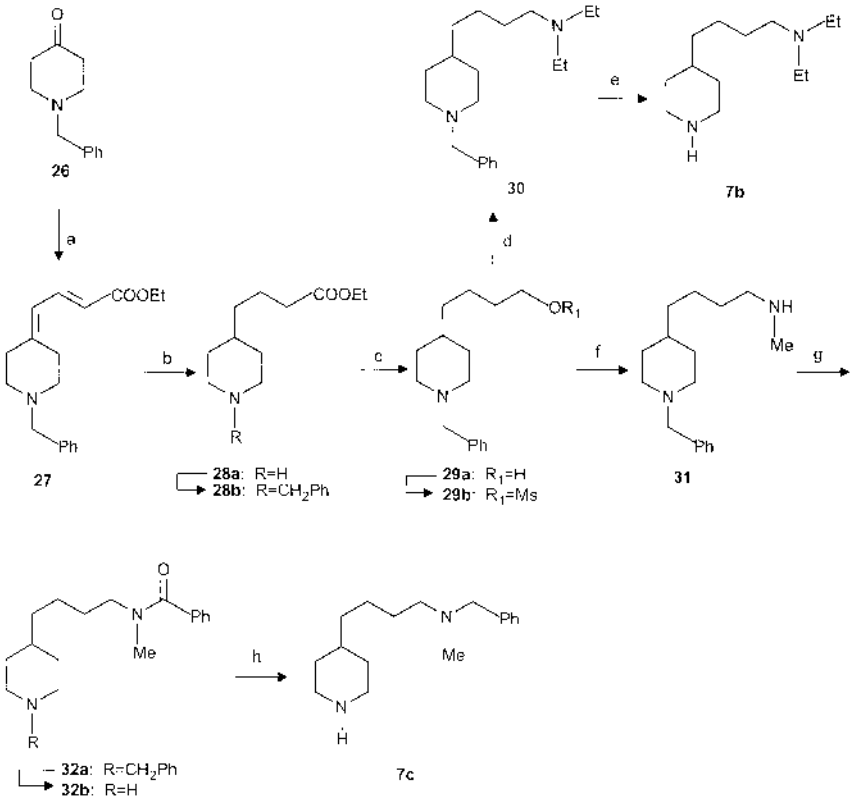
25 5e

Chart 2. Preparation of 10-Phenoxy-5*H*-dibenz[*b*,*f*]azepine **5e**

Pharmacological Results and Discussion

Receptor Binding The muscarinic receptor binding affinities of test compounds were assessed using human recombinant M_1, M_2, M_3 and M_4 receptors. The results, expressed as pK_i values and selectivity ratios for M_2 muscarinic receptors over M_1, M_3 and M_4 muscarinic receptors (M_1/M_2, M_3/M_2 and M_4/M_2, respectively), are presented in Tables 1 and 2. Compound **1** was used as a reference compound.

Initially, the effects of the replacement of the amide bond of the 5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one ring of **3** with the olefinic bond were investigated (Table 1). Compound **8** displayed a strong affinity for M_2 receptors (K_i=2.6±1.1 nM) with an extreme selectivity *versus* the other receptors subtypes (119-, 112- and 39-fold for M_1, M_3 and M_4 receptors,



reagents: a) $(EtO)_2P(O)CH_2CH=CHCOOEt$, EtOH, EtONa, r.t. b) i)H_2, Pd/C 10%, EtOH, r.t. ii)$PhCH_2Cl$, Na_2CO_3, DMF, r.t. c) i)$LiAlH_4$, THF, N_2, 0°C ii)MsCl, Et_3N, THF, 0°C d) Et_2NH, CH_3CN, Δ e) $HCOONH_4$, Pd/C 10%, MeOH, Δ, N_2 f) $MeNH_2$ 40%, CH_3CN, r.t. g) i)PhCOCl, NaOH 1N, CH_3COCH_3, r.t. ii)$HCOONH_4$, Pd/C 10%, MeOH, Δ, N_2 h) $LiAlH_4$, THF,N_2, Δ.

Chart 3. Preparation of Piperidine Derivatives **7b**—**c**

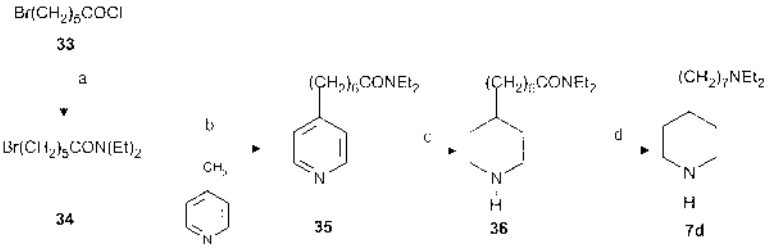


Chart 4. Preparation of Piperidine Derivative **7d**

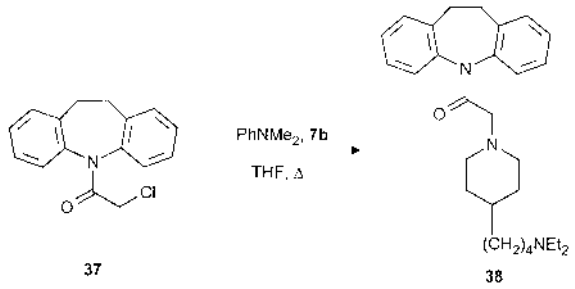


Chart 5. Preparation of Iminodibenzyl Derivative **38**

respectively). For reference, pK_i of compound **3** are 8.40, 9.52, 7.96 and 8.70 for M_1, M_2, M_3 and M_4 receptors, respectively, the selectivity ratios for M_1/M_2 M_3/M_2 and M_4/M_2 being 13, 37 and 7, respectively.[34)]

A comparison of **9**—**11** demonstrated that alkoxy substitution at position 11 of the dibenz[*b*,*f*]azepine skeleton decreases the affinity for M_2 receptors proportionally to the steric hinderance of the chain (BuO **11**<EtO **10**<MeO **9**). The introduction in this position of a phenoxy moiety (**12**) further decreases the affinity for the M_2 receptors. Saturation of the olefinic bond (**38**) reduces the activity on M_2 receptors, although selectivity for the other receptor subtypes remains high.

We next studied the influence of the side chain on the dibenz[*b*,*f*]azepine skeleton (Table 2). A comparison of compounds **8**, **13** and **14** indicates that the highest M_2 affinity and selectivity is obtained with 4 carbon atoms in the (diethylamino)alkyl chain at position 4 of the piperidine ring. Replacement of the terminal diethylamino moiety with a *N*-benzyl-*N*-methyl group resulted in a reduction of the activity for both the piperidine (**15**) and linear alkyl derivatives (**22**). An attempt to distance the piperidine ring from dibenz[*b*,*f*]-azepine did not increase the affinity for the M_2 receptors, and strongly decreased the selectivity (**16**—**17**). Opening the piperidine ring and eliminating the nitrogen atom decreased the activity on M_2 receptors (**19**). Shortening the resulting aliphatic chain did not improve the activity (**20** and **21**). Cyclization of the diethylamino moiety of **21** in a piperidine (**23**) or *N*-methylpiperazine (**24**) ring didn't significantly increase the activity.

***In Vitro* Functional Activity** Functional activities of compounds **8**, **9** and **38** on muscarinic receptors were further evaluated in *in vitro* organ preparations. In field stimulated guinea pig left atria, all the three compounds antagonized the methacoline-induced reduction of contractile response, a muscarinic M_2 receptor mediated agonistic effect. Increasing concentrations of the compounds produced a right shift of the concentration–response curves of the relaxing effects produced by methacholine. The corresponding pA_2 values for **8**, **9** and **38** were 7.08, 6.20 and 5.61, respectively. The functional activity of **8**, **9** and **38** on M_3 receptors was evaluated in guinea-pig ileum preparations. All three compounds antagonized in a concentration-dependent fashion the acetylcholine-induced contractile response of the guinea-pig ileum preparation. Compounds showed a low potency, with an estimated IC_{50} in the μM range of 0.54, 6.79 and 1.19 μM for **8**, **9** and **38**, respectively. These functional *in vitro* studies confirm that **8**, **9** and **38** have high affinity for M_2 muscarinic receptors and low affinity for M_3 muscarinic receptors.

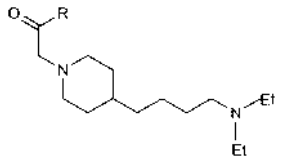
***In Vivo* Antibradycardic Activity** Antibradycardic activity of **8** was evaluated *in vivo* in comparison to AF-DX 116. The effects of **8** on acetylcholine-induced bradycardia in rats were evaluated after intravenous and intraduodenal administration. Acetylcholine administration induced a decrease in heart rate and blood pressure. Both **8** and AF-DX 116 dose-dependently counteracted the acetylcholine-induced bradycardia and hypotension. The calculated ED_{50} for bradycardia of **8** and AF-DX 116 were 55±4 and 13±2 μg/kg, respectively. The corresponding values for hypotension were 80±1 and 56±1 μg/kg, respectively. In the intraduodenal studies, acetylcholine administration induced a dose-dependent reduction of both heart rate (−129±12, −224±12 and −284±10 beats/min after 10, 30 and 100 μg/kg, respectively) and blood pressure (−45±2, −50±2, and −71±4 mmHg, respectively). Compound **8** dose-dependently counteracted both the bradycardia (Fig. 3) and the hypotension induced by acetylcholine. The effects of AF-DX 116 were not significantly different from those of **8**.

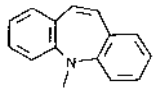
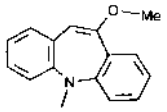
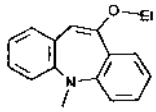
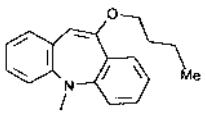
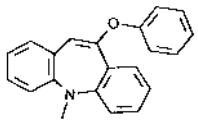
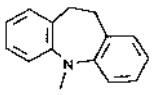
The hemodynamic effects of **8** in dogs were evaluated after oral administration. Figure 4 shows the time courses of mean heart rate for the 24-h period after placebo and two doses of **8**. Compared to the placebo, compound **8** at the dose of 10 mg/kg produced a significant increase in heart rate for the entire observation period.

These *in vivo* studies demonstrated that compound **8** is able to dose-dependently reverse both pharmacologically-induced and physiological bradycardia in different animal models.

***In Vivo* Functional Selectivity** Compound **8** was investigated in rats for potential effects caused by the antagonism of other muscarinic receptor subtypes (mainly M_3). Multiples of the antibradycardic dose were used (15, 50 and 150 mg/

Table 1. Binding Affinities of a Number of (4-Diethylamino)butylpiperidine Derivatives for M_1, M_2, M_3, and M_4 Muscarinic Receptors and Corresponding Selectivity Ratios *versus* M_2 Receptors



Compd. No.	R	Binding affinity, pK_i				Selectivity ratio		
		M_1	M_2	M_3	M_4	M_1/M_2	M_3/M_2	M_4/M_2
8		6.51	8.59	6.54	7.00	119.2	111.5	38.5
9		6.44	7.70	6.38	7.00	18.0	21.0	5.0
10		5.80	7.47	6.25	6.40	47.1	16.5	11.8
11		<6.00	7.23	<6.00	<6.00	>16.9	>16.9	>16.9
12		<6.00	6.92	<6.00	<6.00	>8.3	>8.3	>8.3
38		6.09	7.92	5.96	6.43	67.5	91.7	30.8
1	AF-DX 116	5.54	7.15	5.11	5.92	40.8	108.5	16.9

kg). Studies were carried out in comparison with atropine and AF-DX 116. Mean percent changes, compared to the controls, of the rate of gastric emptying, intestinal transit time, salivary secretion and pupil diameter after the different treatments are listed in Table 3. As expected, atropine significantly delayed the rate of gastric emptying (−84%) and intestinal transit time (−40%), inhibited oxotremorine-stimulated salivary secretion (−54%), and increase pupil diameter (+855%). At the lower dose of 15 mg/kg, compound **8** and AF-DX 116 had no effects of all these functions. At the medium dose (50 mg/kg) AF-DX 116 significantly affected gastric emptying (−74%), intestinal transit (−25%) and pupil diameter (+176%), while **8** only influenced the rate of gastric emptying (−48%). At the highest doses of 150 mg/kg, both compounds delayed gastric emptying and intestinal transit time. At this dose, AF-DX 116 also influenced pupil diameter (+631%), while compound **8** had no effects.

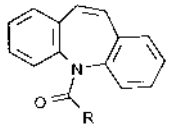
These studies show that in rats the antibradycardic effective doses of **8** have virtual no effects on physiological functions mediated mainly by M_3 and M_1 cholinergic receptors. The compound appears to be more selective than AF-DX 116, especially as far as the pupil diameter is concerned, where a favorable M_1/M_2 selectivity may play an important role.[35]

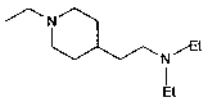
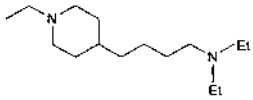
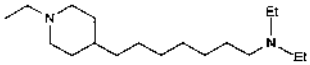
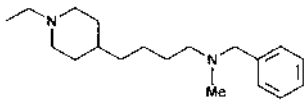
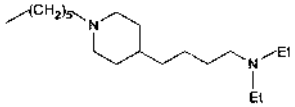
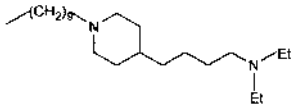
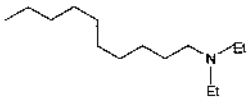
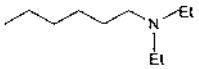
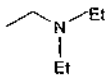
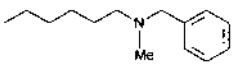
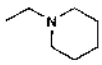
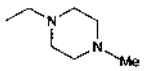
CNS Penetration It is well known that centrally-acting cholinergic agents increase the pain threshold.[36] There is evidence that muscarinic receptors of the M_2 subtype are presynaptic autoreceptors that modify the release of acetylcholine through a negative feedback mechanism. Blocking these receptors by selective antagonists may therefore lead to increased acetylcholine release. Thus, a centrally-acting M_2 antagonist should display significant antinociceptive activity. The central analgesic activity of compounds **8**, **9** and **38** (5 mg/kg s.c.) were evaluated in mice. The study was carried out in comparison with oxotremorine (0.2 mg/kg s.c.) and AF-DX 116 (5 mg/kg s.c.). Mean licking latency before treatments was around 25 s. Oxotremorine produced a significant prolongation of licking latency (70 s), with maximum effect 30—45 min after administration. Both AF-DX 116 and compounds **8**, **9** and **38** produced weak and transient effects (Fig. 5), suggesting poor penetration of the blood brain barrier.

Conclusions

A series of 5*H*-dibenz[*b*,*f*]azepine derivatives having a lateral amino group was synthesized on the basis of bioisosterism of the double bond and amide bond. Among them, **8** (MF 10058) showed a high affinity to the human recombi-

Table 2. Binding Affinities of a Number of 5*H*-Dibenz[*b*,*f*]azepine Derivatives with Different Amine Chains for M_1, M_2, M_3, and M_4 Muscarinic Receptors and Corresponding Selectivity Ratios *versus* M_2 Receptors



Compd. No.	R	Binding affinity, pK_i				Selectivity ratio		
		M_1	M_2	M_3	M_4	M_1/M_2	M_3/M_2	M_4/M_2
13		<6.00	6.44	<6.00	<6.00	>2.8	>2.8	>2.8
8		6.51	8.59	6.54	7.00	119.2	111.5	38.5
14		6.74	7.05	6.34	7.07	2.0	5.1	0.9
15		<6.00	7.26	<6.00	6.60	>18.2	>18.2	4.5
16		7.89	8.05	7.28	8.06	1.4	5.8	1.0
17		7.74	7.85	6.55	7.31	1.1	20.0	3.5
19		6.96	7.42	6.62	6.85	2.9	6.3	3.7
20		<6.00	6.92	<6.00	<6.00	>8.3	>8.3	>8.3
21		<6.00	<6.00	<6.00	<6.00	nt	nt	nt
22		<6.00	7.21	6.32	6.40	16.1	7.7	6.3
23		<6.00	6.92	6.88	7.04	>8.3	1.1	0.8
24		6.62	<6.00	6.64	6.82	<0.2	<0.2	<0.1

nant M_2 receptors (K_i=2.6 nmol/l), a low affinity for the M_4 receptors (39-fold less than for M_2 receptors) and a very low affinity for M_1 and M_3 receptors (119- and 112-fold less than for M_2 receptors). Functional experiments confirmed **8** to be a competitive antagonist with high affinity to the cardiac (pA_2=7.1) and low affinity to the intestinal muscarinic receptors (ED_{50}=0.54 μM). *In vivo* experiments confirmed the *in vitro* M_2 selectivity. Acetylcholine-induced bradycardia was dose-dependently antagonized in rats after both intravenous and intraduodenal administration. In rats, cholinergic functions mediated by M_1 or M_3 receptors (salivary secretion, pupil diameter, gastric emptying, intestinal transit time) were not affected by the oral administration of **8**, even at doses as high as 30 times the antibradycardic effective dose. Nocturnal bradycardia was dose-dependently inhibited in dogs by the oral route, with a duration of action of about 24 h. Compound **8** appears to be a promising cardioselective antimuscarinic agent for the treatment of dysfunctions of the cardiac

conduction system such as sinus or nodal bradycardia ("sick-sinus syndrome") and atrioventricular block.

Experimental

All reagents and solvents were from commercial suppliers and used with no further purification. Proton magnetic resonance spectra (^{1}H-NMR) were recorder on a Bruker AC 200 (4.7 T) instrument. Chemical shifts (δ) are reported in parts per million relative to the internal standard deuterated chloroform (δ=7.26 ppm). The following abbreviations are used: s=singlet, d=doublet, dd=double doublet, td=triple doublet, q=quartet, m=multiplet, br=broad. IR spectra were recorder on a Perkin Elmer Spectrum RX FT-IR System spectrometer in the range 4000—600 cm^{-1}. Reaction products were purified, when necessary, by flash column chromatography on silica gel (J. T. Baker 230—400 mesh). Analytical thin-layer chromatography (TLC) was carried out on precoated glass plates (Macherey-Nagel Durasil-25 UV_{254}), and visualized with UV light at 254 nm and/or I_2 vapor.

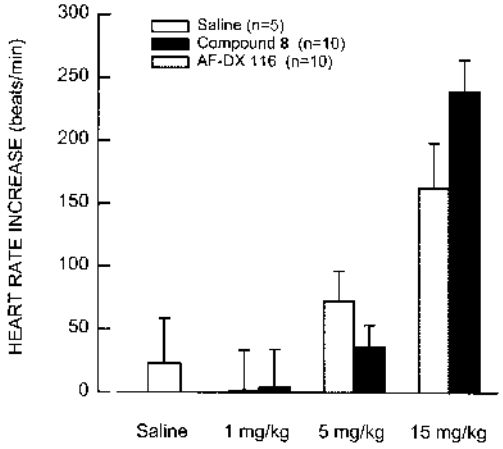


Fig. 3. Dose-Response Effects (Mean±S.E.M.) of Compond **8** and AF-DX 116, Administered by Intraduodenal Route, on the Acetylcholine-Induced Bradycardia in Rat

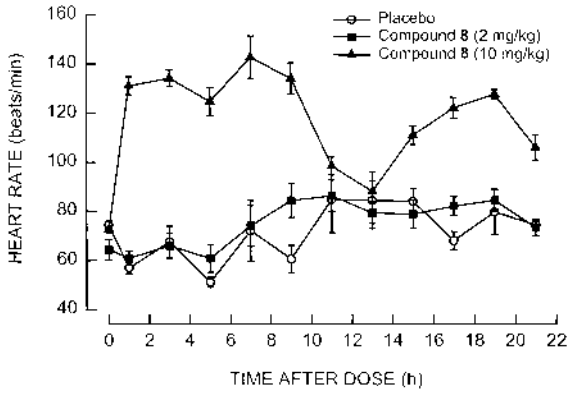


Fig. 4. Time Course of Heart Rate (Mean±S.E.M.) in Two Dogs Receiving Placebo and Different Oral Doses of Compound **8**

10-Phenoxy-5*H*-dibenz[*b*,*f*]azepine (5e) To a solution of phenol (0.43 g, 4.5 mmol) in 8 ml of anhydrous dimethyl sulfoxide was added potassium *t*-butoxide (1.02 g, 4.5 mmol). The solution was stirred at room temperature for 30 min, then 5-acetyl-10-bromo-5*H*-dibenz[*b*,*f*]azepine **25** (0.95 g, 3.0 mmol) was added and the mixture was stirred at 100 °C for 20 h. After cooling to room temperature, the reaction mixture was poured into water (100 ml) and extracted with diethyl ether (4×25 ml). The combined organic phases were washed with 2 N NaOH, dried over Na_2SO_4 and concentrated to give 0.17 g of **5e** as a yellow oil in 20% yield. ^{1}H-NMR ($CDCl_3$) δ: 5.17 (1H, s br, NH), 6.23 (1H, s br, CH=O-Ph), 6.55—7.42 (13H, m).

Ethyl *trans*-4-(*N*-Benzyl-4′-piperidinyliden)-2-butenoate (27) 1-Benzyl-4-piperidone (17.1 ml, 92.2 mmol) and triethyl 4-phosphonocrotonate (25.63 g, 92.2 mmol) were dissolved in 60 ml of absolute ethanol and cooled to 0—5 °C, under a nitrogen atmosphere. Metallic sodium (2.76 g, 119.9 mmol) dissolved in 150 ml of absolute ethanol was added dropwise to the cooled solution at below 5 °C for 40 min. The solution was stirred at 5 °C for 45 min, then kept for 1 h at room temperature. The reaction mixture was diluted with 600 ml of brine and extracted with diethyl ether (5×100 ml). The combined extracts werb washed with water, dried over Na_2SO_4, the solvent was evaporated *in vacuo*, and the residue purified by flash column chromatography eluting with petroleum ether–Et_2O (1 : 1, v/v) to afford 16.6 g of **27** as a yellow oil in 63% yield. IR (neat) cm^{-1}: 2920, 2800, 1710 (C=O), 1635, 1610. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.0 Hz), 2.25—2.60 (8H, m), 3.51 (2H, s), 4.19 (2H, q, J=7.0 Hz), 5.80 (1H, d, J=15.2 Hz, CH–COOEt), 5.96 (1H, d, J=10.9 Hz, CH–CH=CH–COOEt), 7.20—7.35 (5H, m), 7.56 (1H, dd, J=15.2, 10.9 Hz, CH=CH–COOEt).

Ethyl 4-(4′-Piperidinyl)butanoate (28a) A suspension of **27** (16.48 g, 57.7 mmol) and 1.6 g of 10% palladium on activated carbon in 150 ml of ethanol was hydrogenated at atmospheric pressure and room temperature for 8 h. The reaction mixture was filtered through Celite®, and the solvent was evaporated off under reduced pressure. The residue was dissolved in 100 ml of diethyl ether and the solvent was washed with brine, dried over Na_2SO_4,

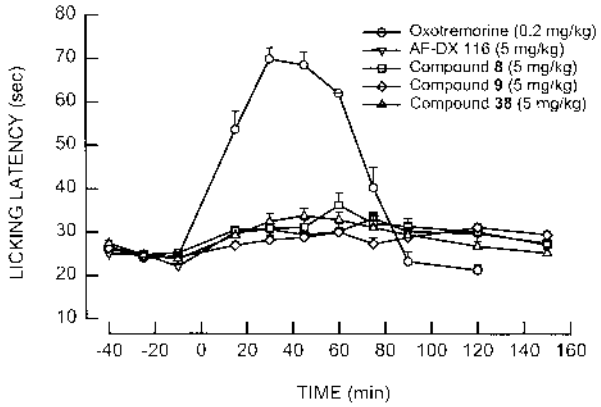


Fig. 5. Licking Latencies (Mean±S.E.M.) after Subcutaneous Administration of Oxotremorine (0.2 mg/kg), AF-DX 116 (5 mg/kg) and Compounds **8**, **9**, and **38** (5 mg/kg) to Groups of 12—17 Mice

Table 3. Percent Changes (Mean±S.E.M.) Compared to Controls of Rate of Gastric Emptying, Intestinal Transit Time, Salivary Secretion and Pupil Diameter after Atropine and Different Doses of AF-DX 116 and Compound **8**

	Gastric emptying	Intestinal transit	Salivary secretion	Pupil diameter
Atropine	−84±10**	−40±6**	−54±7**	855±17**
AF-DX 116				
15 mg/kg	0±8	6±5	−22±8	12±5
50 mg/kg	−74±11**	−25±8*	−18±9	176±67*
150 mg/kg	−87±13**	−36±5**	−12±9	631±90**
Compound **8**				
15 mg/kg	7±9	−2±5	2±10	49±33
50 mg/kg	−48±19**	−19±8	−13±13	4±2
150 mg/kg	−107±14**	−31±9**	−5±12	7±8

* $p<0.05$ *vs.* controls; ** $p<0.01$ *vs.* controls.

Table 4. Physical Data for ϖ-Haloacyldibenzazepine Derivatives **6a**—**g** and **37**

Compd. (No.)	R	*n*	X	Yield (%)	^{1}H-NMR δ (in $CDCl_3$, *J* in Hz)	Ref.
6a	H	1	Cl	>98	3.72 (1H, d, *J*=12.3 Hz, COCHH′Cl), 3.96 (1H, d, *J*=12.3 Hz, COCHCHH′Cl), 6.95 (2H, dd, *J*=15.3, 11.5 Hz, CH=CH), 7.31—7.50 (8H, m)	40)
6b	H	5	Br	96	1.20—1.81 (6H, m, $-(CH_2)_3-$), 1.92 (1H, m, COCHH′), 2.23 (1H, m, COCHH′), 3.32 (2H, t, *J*=6.9, $-CH_2-$Br), 6.95 (2H, dd, *J*=15.2, 12.2 Hz, CH=CH), 7.25—7.50 (8H, m)	—
6c	H	9	Br	94	1.07—2.03 (15H, m, $-(CH_2)_7-$ and COCHH′), 2.20 (1H, m, COCHH′), 3.38 (2H, t, *J*=6.8 Hz, $-CH_2-$Br), 6.95 (2H, dd, *J*=16.3, 13.0 Hz), 7.30—7.55 (m, 8H)	—
6d	OMe	1	Cl	>98	3.73 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 3.80 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 3.88 (3H, d, *J*=4.5 Hz, OCH_3), 3.97 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 4.03 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 6.15 (1H, d, *J*=13.8 Hz, CH=C–OMe), 7.26—7.55 (7H, m), 7.77 (1H, t br, *J*=7.7 Hz)	—
6e	OEt	1	Cl	>98	1.48 (3H, m, CH_2CH_3), 3.73 (0.5H, d, *J*=6.1 Hz, COCHH′Cl), 3.80 (0.5H, d, *J*=6.1 Hz, COCHH′Cl), 3.94—4.17 (3H, m, CH_2CH_3 and COCHH′Cl), 6.14 (1H, d, *J*=11.5 Hz, CH=C–OEt), 7.26—7.55 (7H, m), 7.79 (1H, t br, *J*=7.3 Hz)	—
6f	OBu	1	Cl	94	1.02 (3H, t, *J*=6.8 Hz, $O(CH_2)_3CH_3$), 1.48—1.67 (2H, m, $O(CH_2)_2CH_2CH_3$), 1.74—1.95 (2H, m, $OCH_2CH_2CH_2CH_3$), 3.73 (0.5H, d br, *J*=6.11 Hz, COCHH′Cl), 3.89 (0.5H, d br, *J*=6.11 Hz, COCHH′Cl), 3.90—4.09 (3H, m, CH=C–$OCH_2(CH_2)_2CH_3$ and COCHH′Cl), 7.26—7.55 (7H, m), 7.77 (1H, m)	—
6g	OPh	1	Cl	65	3.82 (1H, d, *J*=13.3 Hz, COCHH′Cl), 4.02 (1H, d, *J*=13.3 Hz, COCHH′Cl), 6.58 (1H, s, CH=C–OPh), 6.95—7.55 (12H, m), 7.77 (1H, dd, *J*=22.2, 7.5 Hz)	—
37	—	—	—	>98	2.85 (2H, m, CHH′–CHH′), *ca.* 3.4 (2H. m br, CHH′–CHH′), 4.02 (2H, dd, *J*=18.7, 11.5 Hz, COCHH′Cl), 7.11—7.40 (8H, m)	41)

filtered, and evaporated *in vacuo* to give 11.2 g of **28a** as a yellow oil in 97% yield. IR (neat) cm^{-1}: 3280 (NH), 2910, 2840, 1725 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, *J*=7.0 Hz), 1.55—1.78 (9H, m), 2.25 (2H, t, *J*=6.8 Hz), 2.53 (2H, td, *J*=11.6, 2.6 Hz, NCH_{ax}), 3.01 (2H, d, *J*=11.6 Hz, NCH_{eq}), 4.09 (2H, q, *J*=6.8 Hz).

Ethyl 4-(*N*-Benzyl-4′-piperidinyl)butanoate (28b) Sodium carbonate (5.9 g, 55.4 mmol), benzylchloride (6.4 ml, 55.4 mmol) and a catalytic amount of sodium iodide were added to **28a** (11.04 g, 55.4 mmol) dissolved in 60 ml of dimethylformamide. The reaction mixture was stirred at room temperature for 6 h, diluted in 400 ml of 0.1 N HCl and extracted with diethyl ether (3×80 ml). The aqueous layer was basified with 40% aqueous NaOH and extracted with diethyl ether (4×100 ml). The combined organic phases were washed with water, dried over Na_2SO_4, filtered and evaporated to give 13.85 g of **28b** as a yellow oil in 86% yield. IR (neat) cm^{-1}: 2927, 2840, 2800, 1736 (C=O), 1454. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.35 (8H, m), 1.50—1.70 (4H, m), 1.91 (2H, t br *J*=10.4 Hz, NCH_{ax}), 2.27 (2H, t, *J*=7.1 Hz), 2.86 (2H, d, *J*=11.2 Hz, NCH_{eq}), 3.46 (2H, s, CH_2Ph), 4.11 (2H, q, *J*=6.8 Hz), 7.20—7.35 (5H, m).

4-(*N*-benzyl-4′-piperidinyl)-1-butanol (29a) A solution of **28b** (13.8 g, 47.8 mmol) in 60 ml of anhydrous tetrahydrofuran was added dropwise, under a nitrogen atmosphere, to a suspension of lithium aluminium hydride (2.7 g, 71.7 mmol) in 40 ml of anhydrous tetrahydrofuran while maintaining the reaction temperature below 10 °C. The mixture was stirred for 1 h at room temperature, cooled to 0 °C and then hydrolyzed by the addition of 1 N NaOH. The resulting suspension was filtered and the filtrate evaporated. The residue dissolved in 100 ml of diethyl ether was washed with water and the organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuo* to give 11.3 g of **29a** as yellow oil in 96% yield. IR (neat) cm^{-1}: 3339 (O–H), 2928, 1454. ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.70 (11H, m), 1.91 (2H, t br *J*=11.1 Hz, NCH_{ax}), 2.86 (2H, d, *J*=10.9 Hz, NCH_{eq}), 3.47 (2H, s, CH_2Ph), 3.61 (2H, t, *J*=5.7 Hz), 7.20—7.35 (5H, m).

4-[4-(Diethylamino)butyl]-*N*-benzylpiperidine (30) Triethylamine (6.4 ml, 45.8 mmol) and **29a** (11.23 g, 45.4 mmol) were dissolved in 60 ml of tetrahydrofuran and cooled to 0 °C. Methanesulfonyl chloride (3.6 ml, 45.8 mmol) dissolved in 20 ml of tetrahydrofuran was added dropwise while the reaction temperature was maintained below 5 °C. The mixture was stirred for 45 min at this temperature and then warmed to room temperature. The resulting suspension was diluted with 60 ml of diethyl ether and the insoluble precipitate was filtered. The solvent was evaporated *in vacuo* to give 15.20 g of **29b** as a yellow oil. The compound was characterized only by IR and reacted immediately. IR (neat) cm^{-1}: 2928, 1454, 1355 (ν_{as} SO_2), 1176 (ν_s SO_2). Methanesulfonate derivative **29b** (15.03 g, 46.2 mmol) and diethylamine (19.1 ml, 184.8 mmol) dissolved in 130 ml of acetonitrile were refluxed for 6 h. After cooling to room temperature, the mixture was diluted with 250 ml of brine and extracted with diethyl ether (4×80 ml). The combined organic phases were washed with water, dried over Na_2SO_4, filtered and evaporated *in vacuo* to give 12.94 g of **30** as a brownish oil in 94% yield. IR (neat) cm^{-1}: 2928, 2840, 2790, 1454. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (6H, t, *J*=7.0 Hz), 1.10—1.70 (11H, m), 1.91 (2H, m, NCH_{ax}), 2.38 (2H, t, *J*=8.4 Hz), 2.50 (4H, q, *J*=7.0 Hz), 2.85 (2H, d br, *J*=10.0 Hz, NCH_{eq}), 3.46 (2H, s, CH_2Ph), 7.20—7.35 (5H, m).

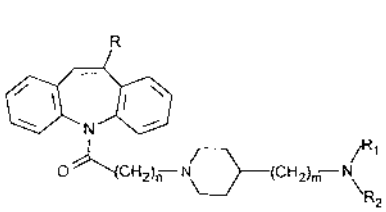
4-[4-(Diethylamino)butyl]piperidine (7b) A mixture of **30** (12.9 g, 42.7 mmol), ammonium formate (13.5 g, 213.5 mmol) and 10% palladium on activate carbon (13 g) in 160 ml of anhydrous methanol was refluxed for 30 min under nitrogen atmosphere. After cooling to room temperature, the reaction mixture was filtered through Celite® and the solvent evaporated off to afford 8.13 g of **7b** as a yellow oil in 90% yield. IR (neat) cm^{-1}: 3278 (N–H), 2929, 1466, 1446. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (6H, t, *J*=7.1 Hz), 1.05—1.75 (12H, m), 2.38 (2H, t, *J*=8.1 Hz), 2.50 (4H, q, *J*=7.1 Hz), 2.55 (2H, m, NCH_{ax}), 3.02 (2H, dt, *J*=12.2, 2.8 Hz, CH_{eq}).

5-(Chloroacetyl)-10-methoxy-5*H*-dibenz[*b*,*f*]azepine (6d) To a solution of 10-methoxy-5*H*-dibenz[*b*,*f*]azepine **5b** (4 g, 20.7 mmol) in tetrahydrofuran (40 ml) were added *N*,*N*-dimethylaniline (2.75 ml, 21.7 mmol) and chloroacetylchloride (1.73 ml, 21.7 mmol), and the solution was refluxed for 1 h. The reaction was quenched with 200 ml of brine and extracted with diethyl ether (3×80 ml). The combined organic phases were washed with 0.1 N HCl (2×100 ml) in order to eliminate *N*,*N*-dimethylaniline, with 0.1 N NaOH (2×100 ml) to eliminate chloroacetylacetic acid, then with water. The organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuo* to give 5.67 g of **6d** as a yellowish powder in quantitative yield. IR (nujol) cm^{-1}: 3040, 1670 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 3.73 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 3.80 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 3.88 (3H, d, *J*=4.5 Hz, OCH_3), 3.97 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 4.03 (0.5H, d, *J*=5.1 Hz, COCHH′Cl), 6.15 (1H, d, *J*=13.8 Hz, CH=C–OMe), 7.26—7.55 (7H, m), 7.77 (1H, t br, *J*=7.7 Hz).

Compounds **6a**—**c** and **6e**—**g** were prepared in the same fashion as described for **6d**. Physical data for these compounds are listed in Table 4.

5-{4-[4-(Diethylamino)butyl]-1-piperidinyl}acetyl-5*H*-dibenz[*b*,*f*]-azepine (8) Sodium carbonate (1.36 g, 12.8 mmol), **6a** (3.46 g, 12.8 mmol) and a catalytic amount of sodium iodide were added to 4-[4-(diethylamino)-butyl] piperidine **7b** (3 g, 14.1 mmol) in 70 ml of acetonitrile. The mixture was refluxed for 1.5 h, diluted in 150 ml of 2 N HCl, and extracted with diethyl ether (2×80 ml). The aqueous layer was basified with 40% aqueous NaOH and extracted with diethyl ether (3×80 ml). The combined organic phases were washed with water, dried over Na_2SO_4, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography elution with petroleum ether–acetone–triethyl amine (16 : 4 : 0.5, v/v/v). After solvent evaporation, the residue was dissolved in diethyl ether and washed with water (3×100 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated *in vacuo* to give 5.04 g of **8** as a light yellow oil in 88% yield. IR (neat) cm^{-1}: 3024, 2929, 1676 (C=O), 1490, 1462, 1442. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (6H, t, *J*=6.9 Hz, NCH_2CH_3), 1.05—2.00 (13H, m, CH_2, CH and NCH_{ax}), 2.37 (2H, m, CH_2NEt_2), 2.50 (4H, q, *J*=6.5 Hz, NCH_2CH_3), 2.68 (2H, d, *J*=9.8 Hz, NCH_{eq}). 2.76 (1H, d, *J*=14.3 Hz, COCHH′), 3.07 (1H, d,

Table 5. Analytical and Spectral Data for Compounds **8**—**17**, **19**—**24** and **38**



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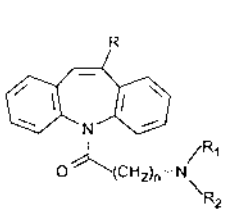

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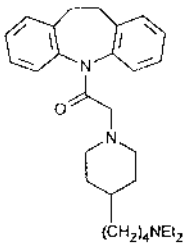

38

Compd. (No.)	R	R_1	R_2	*n*	*m*	Formula	Analysis (%) Calcd (Found) C	H	N	Yield (%)	^{1}H-NMR δ (in $CDCl_3$)
8	H	Et	Et	1	4	$C_{29}H_{39}N_3O$	78.16 (78.06	8.82 8.90	9.43 9.37)	88	0.99 (6H, t, *J*=6.9 Hz, $NCH_2C\underline{H}_3$), 1.05—2.00 (13H, m, $C\underline{H}_2$, C<u>H</u> and $NC\underline{H}_{ax}$), 2.37 (2H, m, $C\underline{H}_2NEt_2$), 2.50 (4H, q, *J*=6.5 Hz, $NC\underline{H}_2CH_3$), 2.68 (2H, d, *J*=9.8 Hz, $NC\underline{H}_{eq}$). 2.76 (1H, d, *J*=14.3 Hz, $COC\underline{H}H'$), 3.07 (1H, d, *J*=14.3 Hz, $COCH\underline{H}'$), 6.91 (2H, dd, *J*=13.7, 11.7 Hz, CH=CH), 7.30—7.50 (8H, m)
9	OMe	Et	Et	1	4	$C_{30}H_{41}N_3O_2$	75.75 (75.61	8.69 8.70	8.83 8.78)	92	1.00 (6H, t, *J*=7.0 Hz, $NCH_2C\underline{H}_3$), 1.08—2.03 (13H, m, CH_2, CH and $NC\underline{H}_{ax}$), 2.39 (2H, t, *J*=7.1 Hz, $C\underline{H}_2NEt_2$), 2.52 (4H, q, *J*=7.0 Hz, $NC\underline{H}_2CH_3$), 2.60—3.15 (4H, m, $COCH_2N$ and $NC\underline{H}_{eq}$), 3.86 (3H, d, *J*=3.8 Hz, OCH_3), 6.11 (1H, d, *J*=7.3 Hz, $C\underline{H}$=$COCH_3$), 7.20—7.48 (7H, m), 7.72 (1H, m)
10	OEt	Et	Et	1	4	$C_{31}H_{43}N_3O_2$	76.03 (75.88	8.85 8.92	8.58 8.60)	67	1.00 (6H, t, *J*=6.9 Hz, $NCH_2C\underline{H}_3$), 1.08—2.05 (16H, m, CH_2, CH, $OCH_2C\underline{H}_3$ and $NC\underline{H}_{ax}$), 2.37 (2H, t, *J*=7.1 Hz, $C\underline{H}_2NEt_2$), 2.49 (4H, q, *J*=6.9 Hz, $NC\underline{H}_2CH_3$), 2.60—3.15 (4H, m, $COCH_2N$ and $NC\underline{H}_{eq}$), 4.05 (2H, q br, $OC\underline{H}_2CH_3$), 6.11 (1H, d, *J*=5.1 Hz, C<u>H</u>=C–OEt), 7.20—7.46 (7H, m), 7.75 (1H, m)
11	OBu	Et	Et	1	4	$C_{33}H_{47}N_3O_2$	76.55 (76.53	9.15 9.20	8.12 8.10)	71	1.00 (9H, m, $NCH_2C\underline{H}_3$ and $O(CH_2)_3C\underline{H}_3$), 1.05—2.05 (17H, m, CH_2, CH, $OCH_2(C\underline{H}_2)_2CH_3$ and $NC\underline{H}_{ax}$), 2.37 (2H, t, *J*=6.9 Hz, $C\underline{H}_2NEt_2$), 2.49 (4H, q, *J*=6.6 Hz, $NC\underline{H}_2CH_3$), 2.60—3.15 (4H, m, $COCH_2N$ and $NC\underline{H}_{eq}$), 4.00 (2H, s br, $OC\underline{H}_2(CH_2)_2CH_3$), 6.11 (1H, m, C<u>H</u>=COBu), 7.15—7.48 (7H, m), 7.72 (1H, m)
12	OPh	Et	Et	1	4	$C_{35}H_{43}N_3O_2$	78.12 (77.99	8.06 8.10	7.81 7.75)	67	1.00 (6H, t, *J*=6.8 Hz, $NCH_2C\underline{H}_3$), 1.05—2.10 (13H, m, CH_2, CH and $NC\underline{H}_{ax}$), 2.38 (2H, t, *J*=6.9 Hz, $C\underline{H}_2NEt_2$), 2.51 (4H, q, *J*=6.8 Hz, $NC\underline{H}_2CH_3$), 2.60—2.96 (3H, m, $COC\underline{H}H'$ and $NC\underline{H}_{eq}$), 3.17 (1H, m, $COCH\underline{H}'$), 6.54 (1H, d, *J*=11.6 Hz, C<u>H</u>=C–OPh), 6.95—7.49 (12H, m), 7.72 (1H, dd, *J*=23.1, 7.4 Hz)
13	H	Et	Et	1	2	$C_{27}H_{35}N_3O$	77.66 (77.50	8.45 8.50	10.06 10.00)	77	1.00 (6H, t, *J*=6.8 Hz, $NCH_2C\underline{H}_3$), 1.05—2.05 (9H, m, CH_2, CH and $NC\underline{H}_{ax}$), 2.39 (2H, m, $C\underline{H}_2NEt_2$), 2.49 (4H, q, *J*=6.8 Hz, $NC\underline{H}_2CH_3$), 2.65 (2H, d, *J*=9.0 Hz, $NC\underline{H}_{eq}$), 2.78 (1H, d, *J*=14.7 Hz, $COC\underline{H}H'$), 3.07 (1H, d, *J*=14.7 Hz, $COCH\underline{H}'$), 6.92 (2H, dd, *J*=13.5, 11.1 Hz, CH=CH), 7.30—7.45 (8H, m)
14	H	Et	Et	1	7	$C_{32}H_{45}N_3O$	78.80 (78.75	9.30 9.31	8.62 8.59)	61	1.00 (6H, t, *J*=7.1 Hz, $NCH_2C\underline{H}_3$), 1.05—2.05 (19H, m, CH_2, CH and $NC\underline{H}_{ax}$), 2.37 (2H, m, $C\underline{H}_2NEt_2$), 2.49 (4H, q, *J*=7.1 Hz, $NC\underline{H}_2CH_3$), 2.65 (2H, d, *J*=9.7 Hz, $NC\underline{H}_{eq}$), 2.78 (1H, d, *J*=15.1 Hz, $COC\underline{H}H'$), 3.07 (1H, d, *J*=15.1 Hz, $COCH\underline{H}'$), 6.92 (2H, dd, *J*=12.5, 11.0 Hz, CH=CH), 7.30—7.45 (8H, m)
15	H	Bn	Me	1	4	$C_{33}H_{39}N_3O$	80.28 (80.07	7.96 8.00	8.51 8.49)	95	1.00—2.00 (13H, m, CH_2, CH and $NC\underline{H}_{ax}$), 2.15 (3H, s, NCH_3), 2.32 (2H, t, *J*=6.8 Hz, $C\underline{H}_2$NMeBn), 2.66 (2H, d, *J*=9.1 Hz, $NC\underline{H}_{eq}$), 2.76 (1H, d, *J*=14.3 Hz, $COC\underline{H}H'$), 3.07 (1H, d, *J*=14.3 Hz, $COCH\underline{H}'$), 3.44 (2H, s, $NC\underline{H}_2Ph$), 6.92 (2H, dd, *J*=15.7, 11.8 Hz, CH=CH), 7.20—7.45 (8H, m)
16	H	Et	Et	5	4	$C_{33}H_{47}N_3O$	78.99 (78.80	9.44 9.55	8.37 8.25)	71	1.00 (6H, t, *J*=6.8 Hz, $NCH_2C\underline{H}_3$), 1.08—1.98 (20H, m, CH_2, CH, $COC\underline{H}H'$ and $NC\underline{H}_{ax}$), 2.20 (3H, m, $COCH\underline{H}'$ and $C\underline{H}_2N(CH_2CH_2)_2CH$), 2.38 (2H, t, *J*=6.9 Hz, $C\underline{H}_2NEt_2$), 2.50 (4H, q, *J*=6.8 Hz, $NC\underline{H}_2CH_3$), 2.82 (2H, d br, *J*=10.2 Hz, $NC\underline{H}_{eq}$), 6.92 (2H, dd, *J*=15.8, 11.3 Hz, CH=CH), 7.27—7.45 (8H, m)
17	H	Et	Et	9	4	$C_{37}H_{55}N_3O$	79.66 (79.45	9.94 9.99	7.53 7.50)	34	1.00 (6H, t, *J*=7.0 Hz, $NCH_2C\underline{H}_3$), 1.07—2.00 (28H, m, CH_2, CH, $COC\underline{H}H'$ and $NC\underline{H}_{ax}$), 2.20 (3H, m, $COCH\underline{H}'$ and $CH_2N(CH_2CH_2)_2CH$), 2.40 (2H, t, *J*=7.0 Hz, CH_2NEt_2), 2.51 (4H, q, *J*=7.0 Hz, $NC\underline{H}_2CH_3$), 2.88 (2H, d br, *J*=10.6 Hz, $NC\underline{H}_{eq}$), 6.92 (2H, dd, *J*=16.5, 11.3 Hz, CH=CH), 7.27—7.45 (8H, m)

Table 5. Continued

Compd. (No.)	R	R_1	R_2	*n*	*m*	Formula	Analysis (%) Calcd (Found) C	H	N	Yield (%)	^{1}H-NMR δ (in $CDCl_3$)
19	H	Et	Et	9	—	$C_{28}H_{38}N_2O$	80.34 (80.02	9.15 9.19	6.69 6.58)	38	1.00 (6H, t, *J*=7.0 Hz, $NCH_2C\underline{H}_3$), 1.07—1.60 (14H, m, $COCH_2(CH_2)_7$), 1.90 (1H, m, $COC\underline{H}H'$), 2.19 (1H, m, $COCH\underline{H}'$), 2.37 (3H, t, *J*=7.3 Hz, $C\underline{H}_2NEt_2$), 2.50 (4H, q, *J*=7.0 Hz, $NC\underline{H}_2CH_3$), 6.92 (2H, dd, *J*=16.6 Hz, 11.5, $C\underline{H}$=$C\underline{H}$), 7.27—7.50 (8H, m)
20	H	Et	Et	5	—	$C_{24}H_{30}N_2O$	79.52 (79.11	8.34 8.31	7.73 7.80)	45	0.97 (6H, t, *J*=7.0 Hz, $NCH_2C\underline{H}_3$), 1.15—1.60 (6H, m, $COCH_2(CH_2)_3$), 1.90 (1H, m, $COC\underline{H}H'$), 2.16 (1H, m, $COCH\underline{H}'$), 2.31 (3H, t, *J*=7.0 Hz, $C\underline{H}_2NEt_2$), 2.45 (4H, q, *J*=7.0 Hz, $N(C\underline{H}_2CH_3)_2$), 6.95 (2H, dd, *J*=16.1, 11.6 Hz, $C\underline{H}$=$C\underline{H}$), 7.30—7.45 (8H, m)
21	H	Et	Et	1	—	$C_{20}H_{22}N_2O$	78.40 (78.21	7.24 7.19	9.14 9.09)	91	0.87 (6H, t, *J*=7.2 Hz, $NCH_2C\underline{H}_3$), 2.53 (4H, qd, *J*=7.3, 2.5 Hz, $NC\underline{H}_2CH_3$), 2.94 (1H, d, *J*=15.6 Hz, $COCH\underline{H}'$), 3.23 (1H, d, *J*=15.6 Hz, $COCH\underline{H}'$), 6.95 (2H, dd, *J*=15.8, 11.2 Hz, $C\underline{H}$=$C\underline{H}$), 7.30—7.45 (8H, m)
22	H	Bn	Me	5	—	$C_{28}H_{30}N_2O$	81.91 (81.94	7.37 7.45	6.82 6.74)	73	1.12—1.58 (6H, m, $COC\underline{H}_2(CH_2)_3$), 1.92 (1H, m, $COC\underline{H}H'$), 2.13 (3H, s, $NC\underline{H}_3$), *ca.* 2.2 (1H, m, $COC\underline{H}H'$), 2.27 (2H, m, $C\underline{H}_2NCH_3Bn$), 3.43 (2H, s, NCH_2Ph), 6.91 (2H, dd, *J*=16.8, 11.0 Hz, $C\underline{H}$=$C\underline{H}$), 7.23—7.45 (13H, m)
23	H	–$(CH_2)_5$–		1	—	$C_{21}H_{22}N_2O$	79.21 (78.92	6.96 7.12	8.80 8.74)	84	1.23—1.54 (6H, m, –$(CH_2)_3$–), 2.28 (4H, t, *J*=5.4 Hz, CH_2–N–CH_2), 2.78 (1H, d, *J*=15.3 Hz, $COC\underline{H}H'$), 3.05 (1H, d, *J*=15.3 Hz, $COCH\underline{H}'$), 6.93 (2H, d, *J*=1.7 Hz, $C\underline{H}$=$C\underline{H}$), 7.27—7.45 (8H, m)
24	H	N-methyl Piperazine		1	—	$C_{21}H_{23}N_3O$	75.65 (75.01	6.95 7.02	12.60 12.51)	87	2.23 (3H, s, $NC\underline{H}_3$), 2.38 (8H, s br, $N[C\underline{H}_2C\underline{H}_2]_2N$), 2.76 (1H, d, *J*=14.1 Hz, $COC\underline{H}H'$), 3.08 (1H, *J*=14.1 Hz, $COC\underline{H}H'$), 6.95 (2H, dd, *J*=12.3, 11.1 Hz, $C\underline{H}$=$C\underline{H}$), 7.30—7.45 (8H, m)
38	—	—	—	—	—	$C_{29}H_{41}N_3O$	77.81 (77.58	9.23 9.39	9.17 9.42)	84	1.00 (6H, m, $NCH_2C\underline{H}_3$), 1.05—2.15 (13H, m, CH_2, CH and $NC\underline{H}_{ax}$), 2.37 (2H, m, $C\underline{H}_2NEt_2$), 2.50 (4H, q, *J*=7.1 Hz, $NC\underline{H}_2CH_3$), 2.60—3.55 (8H, m, $COCH_2N$, CH_2–CH_2 and $NC\underline{H}_{eq}$), 7.10—7.45 (8H, m)

J=14.3 Hz, $COCH\underline{H}'$), 6.91 (2H, dd, *J*=13.7, 11.7 Hz, CH=CH), 7.30—7.50 (8H, m).

Compounds **9**—**17**, **19**—**24** and **38** were prepared in the same fashion as described for **8**. Physical data for these compounds are listed in Table 5.

4-[4-(Methylamino)butyl]-*N*-benzylpiperidine (31) To a stirred solution of methanesulfonate derivative **29b** (1.65 g, 5.1 mmol) in acetonitrile (7 ml) was added methylamine 40% in water (102 mmol), and the mixture was stirred for 15 h at room temperature. The reaction was poured in brine (80 ml), extracted with diethyl ether (3×50 ml), dried over Na_2SO_4, and the solvent was removed *in vacuo* to afford 1.35 g of **31** as a yellow oil in 98% yield. IR (neat) cm^{-1}: 3250 (NH), 2900, 2830, 2780, 1440. ^{1}H-NMR ($CDCl_3$) δ: 1.14—1.70 (11H, m), 1.90 (2H, t br, *J*=11.5 Hz, $NC\underline{H}_{ax}$), 2.41 (3H, s), 2.54 (2H, *J*=7.1 Hz, t), 2.85 (2H, d br, *J*=11.4 Hz, $NC\underline{H}_{eq}$), 3.46 (2H, s, $C\underline{H}_2Ph$), 7.20—7.35 (5H, m).

4-[4-(*N*-Benzoyl-*N*-methylamino)butyl]-1-benzylpiperidine (32a) To a stirred solution of **31** (1.30 g, 5.0 mmol) in acetone (7 ml) was added 1 N NaOH (5.5 mmol), then a solution of benzoyl chloride (0.61 ml, 5.25 mmol) in acetone (3 ml) was added dropwise. The reaction mixture was stirred at room temperature for 2 h and then poured in brine (20 ml), made alkaline with 1 N NaOH and extracted with diethyl ether (3×20 ml). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo.* The residue was purified by flash column chromatography elution with $CHCl_3$–MeOH (98 : 2, v/v) to afford 1.50 g of **32a** as a light yellow oil in 82% yield. IR (neat) cm^{-1}: 2900, 2840, 1620 (C=O), ^{1}H-NMR ($CDCl_3$) δ: 1.10—2.00 (13H, m), 2.85—3.10 (5H, m), 3.20 (1H, t br, *J*=7.0 Hz), 3.48 (2H, s, $C\underline{H}_2Ph$), 3.51 (1H, m), 7.20—7.35 (5H, m), 7.37 (5H, s).

4-[4-(*N*-Benzoyl-*N*-methylamino)butyl]piperidine (32b) The title compound was prepared as described for **7b**, and was produced in 91% yield. IR (neat) cm^{-1}: 3260 (NH), 2890, 2830, 1615 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.90—1.75 (11H, m), 2.07 (1H, s br, NH), 2.56 (2H, t br, *J*=10.0 Hz, $NC\underline{H}_{ax}$), 2.86—3.10 (5H, m, $NC\underline{H}_{eq}$ e $NC\underline{H}_3$), 3.20 (1H, t br, *J*=5.7 Hz), 3.50 (1H, t br, *J*=5.7 Hz), 7.35 (5H, s).

4-[4-(*N*-Benzyl-*N*-methylamino)butyl]piperidine (7c) The title compound was prepared as described for **29a**, heating at reflux for 1 h, in 92% yield. IR (neat) cm^{-1}: 2910, 2840, 2780, 1450. ^{1}H-NMR ($CDCl_3$) δ: 0.90—1.80 (12H, m), 2.17 (3H, s), 2.34 (2H, t, *J*=6.4 Hz), 2.56 (2H, td, *J*=11.1, 2.8 Hz, $NC\underline{H}_{ax}$), 3.03 (2H, dt, *J*=11.1, 2.8 Hz, $NC\underline{H}_{eq}$), 3.45 (2H, s, $C\underline{H}_2Ph$), 7.20—7.35 (5H, m).

***N*,*N*-Diethyl-6-bromohexanamide (34)** A solution of 6-bromohexanoyl chloride (2.0 g, 9.37 mmol) in acetone (4 ml) was added dropwise to a solution of diethylamine (4.9 ml, 46.85 mmol) in water (8 ml) at 0 °C. The mixture was stirred for 2 h at 0 °C, then poured in brine (40 ml) and extracted with diethyl ether (3×30 ml). The combined extract was washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give 2.2 g of **34** as a yellow oil in 94% yield. IR (neat) cm^{-1}: 2920, 1610 (C=O), 1420. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, *J*=7.0 Hz, $CONCH_2C\underline{H}_3$) 1.14 (3H, t, *J*=7.0 Hz, $CONCH'_2C\underline{H}'_3$), 1.35—1.75 (4H, m), 1.87 (2H, m), 2.29 (2H, t, *J*=7.8 Hz), 3.20—3.46 (6H, m).

***N*,*N*-Diethyl-7-(4-pyridyl)heptanamide (35)** A suspension of 50% sodium amide in toluene (0.75 g, 9.60 mmol) was added to a solution of 4-picoline (0.93 ml, 9.60 mmol) in anhydrous toluene (10 ml) in a nitrogen atmosphere. The mixture was stirred for 30 min at 80 °C, cooled to room temperature, then **34** (1.6 g, 6.40 mmol) in toluene (6 ml) was added. The resulting solution was stirred at 80 °C for 6 h, then poured in brine (30 ml) and the organic layer was separated. The water layer was extracted with diethyl ether (3×20 ml). The combined extracts were washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by a flash chromatography column with petroleum ether–acetone (65 : 35, v/v) to afford 0.51 g of **35** as a yellow oil in 30% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, t, *J*=7.0 Hz), 1.15 (3H, t, *J*=7.0 Hz), 1.27—1.80 (8H, m), 2.26 (2H, t, *J*=7.0 Hz, $C\underline{H}_2CONEt_2$), 2.58 (2H, t, *J*=7.0 Hz, $C\underline{H}_2C_5H_4N$), 3.32 (4H, m,), 7.08 (2H, dd, *J*=4.1, 1.3 Hz), 8.45 (2H, dd, *J*=4.1, 1.6 Hz).

***N*,*N*-Diethyl-7-(4-piperidinyl)heptanamide (36)** Pyridyl derivative **35** (0.47 g) was dissolved in 1.9 M in methanol HCl (20 ml) and stirred at room temperature for 40 min. The solvent was evaporated, the residue was dissolved in acetic acid, and PtO_2 (25 mg) was added. The mixture was maintained under a hydrogen atmosphere at room temperature and atmospheric pressure for 24 h. After the catalyst was filtered off and the filtrate was basified with 32% NaOH, it was then extracted with diethyl ether. The combined extracts were washed with water, dried over Na_2SO_4 and concentrated to af-

ford 0.41 g of **36** in 85% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, J=7.0 Hz), 1.15 (3H, t, J=7.0 Hz), 1.20—1.80 (16H, m), 2.27 (2H, t, J=7.8 Hz, $C\underline{H}_2CONEt_2$), 2.55 (2H, td, J=11.4, 2.2 Hz, $NC\underline{H}_{ax}$), 3.02 (2H, dt, J=11.4, 2.1 Hz, $NC\underline{H}_{eq}$), 3.32 (4H, m, $NC\underline{H}_2CH_3$).

4-[4-(Diethylamino)heptyl]piperidine (7d) The title compound was prepared as described for **29a**, heating at reflux for 2 h, and was produced in 96% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (6H, t, J=6.9 Hz), 1.10—1.80 (18H, m), 2.39 (2H, t, J=7.7 Hz, $C\underline{H}_2NEt_2$), 2.51 (4H, q, J=6.9 Hz, $NC\underline{H}_2CH_3$), 2.55 (2H, m, $NC\underline{H}_{ax}$), 3.03 (2H, dt, J=11.8, 2.8 Hz, $NC\underline{H}_{eq}$).

Receptor Binding Assay Membranes of Hamster ovarian cells expressing human M_1, M_2, M_3 or M_4 receptors were suspended in a 10 mM Tris–HCl solution at pH 7.2 containing 2 mM EDTA (plus 10% sucrose in the case of M_1, M_3 and M_4 receptors). Aliquots of the membrane fractions (0.02 ml) were incubated in duplicate, with 10 increasing concentrations (from 10^{-10} to 10^{-5} M) of the reference (4-DAMP) or test compounds. The radioligand [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) was added to the membrane preparations, which were then incubated for 60 min at 25 °C or 27 °C. Apparent equilibrium dissociation constants (K_d) for [^{3}H]NMS binding were 0.28, 0.29, 0.19, 0.13 nM for M_1, M_2, M_3, M_4 receptors, respectively. Maximum receptor density (B_{max}) was 64, 65, 4400, 1200 fmol/mg protein for M_1, M_2, M_3, M_4 receptors, respectively.

The assay was terminated by rapid filtration under suction using a 96-well harvester. After 4—5 washes with ice-cold buffer, the filters were put in plastic bags with 25 ml scintillant cocktail, and the bags were sealed. The radioactivity was counted using a liquid scintillation counter. Affinity constants (K_i) of the test compounds for muscarinic receptor subtypes were estimated with non-linear regression.

Guinea-Pig Atrium Isolated left atria were prepared from male or female Duncan Hartley derived guinea pigs weighing 325±25 g. Animals were sacrificed by CO_2 overexposure. Each atria was placed under 1 g tension in a 10 ml bath containing McEwen's solution at pH 7.4, bubbled with 95% O_2/5% CO_2 at 32 °C, and subjected to field stimulation by 70% maximum voltage, 2.5 Hz at 0.5 ms pulse width. The left atria was connected to an isometric transducer and two pen recorders and allowed to equilibrate for 60 min before the field stimulation was initiated. Each tissue was accepted for experimental use only if 1 g or more of tension was obtained. A cumulative relaxation–response curve to methacholine was then generated with consecutive applications of 9 concentrations in 3-fold increments ranging from 1 nM to 10 μM at 2-min intervals for a total of 18 min to establish the maximal response. In 4 separate tissues, similar methacholine concentration–response experiments were carried out in the presence of test compound concentration (low, middle and high), following a 15-min incubation period. pA_2 values for the test compounds were calculated by linear regression of the corresponding Schild plots.

Guinea-Pig Ileum Segments (2—3 cm) of proximal ileum were suspended in 30 ml Tyrode solution at 37±1 °C. The preparation was connected to an isometric strain gauge and was kept at a resting tension of 1 g. The changes in tension were registered through a polygraphic recorder. The ileum was stimulated with a sub-maximal concentration of acetylcholine (0.03 μM) every 4—5 min. The drugs were prepared in physiological saline solution. The activity of test compounds under investigation was expressed as the percent inhibition of the contraction induced by acetylcholine. The IC_{50} was calculated by non-linear regression.

Antibradycardic Activity in Rats Male Sprague–Dawley rats weighing 270±5 g were used in the study. Animals (two per cage) were housed for a minimum of 5 d before the experiments under a 12-h light-dark cycle at room temperature (20±2 °C) and 55% minimum humidity. Rats had free access to commercial chow and tap water. Animals were anesthetized with urethane (1.5 g/kg, i.p.) and their body temperature was maintained at 37 °C with a heating pad. A 2 cm incision was made in the middle of the neck and a tracheotomy tube was inserted. The carotid aorta was isolated and connected with a catheter to a pressure transducer. Heart rate and blood pressure were recorded and analyzed with the software HEM (Notocord, Croissy sur Seine, France). Another catheter was introduced in the left jugular vein for acetylcholine perfusion. A third catheter was introduced in the right jugular vein for vehicle, test or reference compound administration. After a 10 min stabilization period, acetylcholine (50 μg/min/kg) was continuously infused intravenously until stabilization of the blood pressure. The vehicle (sterile saline, 1 ml/kg) was administered as an intravenous bolus. Test compounds were then injected as boluses at increasing concentrations (10, 50, 250 μg/kg). Finally, atropine (10 μg/kg) was injected intravenously. At the end of the experiments, rats were killed by urethane overdose. Four animals were used for each dose of the test compounds. ED_{50} values were estimated by non-linear regression.

In the intraduodenal studies, male Sprague–Dawley rats weighing 315± 3 g were housed two per cage under a 12-h light–dark cycle at room temperature (20±2 °C) and 55% minimum humidity for a minimum of 5 d before the experiments. Animals had free access to commercial rat chow and tap water. Rats were anesthetized with urethane (1.5 g/kg, i.p.) and body temperature was maintained at 37 °C with a heating pad. A 2 cm incision was made at the middle of the neck and a tracheotomy tube was inserted. A catheter, filled with sterile heparin solution (5 U/ml), was inserted in the carotid aorta and connected to a pressure transducer. Heart rate and blood pressure were recorded and analyzed with the software HEM (Notocord, Croissy sur Seine, France). After a 10 min stabilization period, three boluses of acetylcholine (10, 30 and 100 μg/kg) were administered intravenously at 5 min intervals. Then, vehicle (sterile saline, 1 ml/kg) or test compounds (1, 5 or 15 mg/kg) were administered through a catheter inserted in the duodenum. Forty-five minutes later, a second series of boluses of acetylcholine (10, 30 and 100 μg/kg) were administered intravenously, again at 5 min intervals. At the end of experiments, rats were killed by urethane overdose. Ten animals were used for each dose of test compounds. Five rats were employed for the vehicle administration. Statistical comparisons were carried out using one-way analysis of variance for completed randomized block.

Hemodynamic Effects in Dogs Two beagle dogs (11.5 and 12.4 kg) participated in the study. Animals were housed in individual stainless steel boxes of standard dimensions (1.2 m^2). These animals were placed in an air-conditioned (17—21 °C) animal house kept at relative humidity between 45% and 65% with non-recycled filtered air changed approximately 10 times per hour. The artificial day/night cycle involved 12 h light and 12 h darkness with light on at 7:30 a.m. A telemetric transmitter was implanted under general anesthesia and aseptic conditions for measurement of blood pressure and heart rate. Implantation was performed under anesthesia induced by thiopental (20 mg/kg i.v.) and then halothane (1—1.5%, i.v.). The telemetric transmitter was implanted in the left flank. The electrodes of the transmitters were placed in lead 2. The sensor catheter of transmitters was introduced into the femoral artery. Telemetric transmitters were implanted at least 10 d before use. The measurements were done in the animal room by means of RLA2000 biotelemetry receivers. Using telemetry, the animals remained free to move about during the entire period of measurement. Systolic and diastolic blood pressure and heart rate were measured during 15-s periods at regular intervals of 5 min. Hemodynamic variables were recorded for 24 h after dosing. Any gross behavioral or autonomic changes observed during the experiment were recorded. Placebo and compound **8** (2 and 10 mg/kg) were administered according to a randomized four-way cross-over design with a washout period of at least one week. Treatments were administered orally in gelatin capsules at approximately 10:30 p.m.. Statistical comparisons were carried out using repeated analysis of variance measurements.

Gastric Emptying in Rats Gastric emptying was evaluated by measuring the rate of disappearance on an aqueous suspension of phenol red from the stomach.[37] Compound **8** and AF-DX 116 were examined at doses of 15, 50 and 150 mg/kg by the oral route and administered to groups of 8 animals. Atropine (20 mg/kg) was used as positive control drug. Wistar rats (200—300 g) were used in this study. Animals were housed in cages of standard dimensions with sawdust. The animal house was kept at a temperature of 19—23 °C and a relative humidity of 45—65% with non-recycled filtered air changed approximately 10 times per hour. A 12-h day–night cycle was adopted with light on at 7:30 a.m. Rats received commercial food. Tap water was available *ad libitum* in polycarbonate feeder bottles with a stainless steel nipple. The day prior to the study, animals were kept on a water only fast in cages with grid floors in order to minimize coprophagia. On the study day, animals were dosed as defined by the randomization plan. Sixty minutes after test compound administration, animals were orally given a 1.5 ml suspension of 0.05% phenol red in 2.5% carboxymethylcellulose (kept at 37 °C±0.5). Ten minutes after the administration of phenol red, animals were sacrificed by pentobarbital overdosing (i.p.). Following midline laparotomy and ligature of the pylorus, the stomach was cut longitudinally and placed in 30 ml of a 0.9% NaCl solution. Twenty-four hours afterwards, the stomach was removed and the volume adjusted to 36 ml with 0.9% NaCl, and 4 ml of 1 M NaOH was added. After homogenization, the mixture was centrifuged at 3000 rpm for 10 min. Phenol red concentrations were measured by spectrophotometry at 550 nM.

Intestinal Transit Time in Rats Intestinal transit time was evaluated by measuring the distance covered in the intestines by a suspension of orally administered vegetal charcoal.[38] Compound **8** and AF-DX 116 were examined at doses of 15, 50 and 150 mg/kg administered by the oral route to groups of 8 animals. Atropine (20 mg/kg *p.o.*) was used as a positive control drug. Wistar rats (200—300 g) were used in this study. Animals were housed

in cages of standard dimensions with sawdust. The animal house was kept at a temperature of 19—23 °C and a relative humidity of 45—65% with non-recycled filtered air changed approximately 10 times per hour. A 12-h day–night cycle was adopted (7:30 a.m.—7:30 p.m.). Rats received commercial food. Tap water was available *ad libitum* in polycarbonate feeder bottles with a stainless steel nipple. The day prior to the study, animals were kept on a water only fast in cages with grid floors in order to minimize coprophagia. On the study day, animals were dosed as defined by the randomization plan. Sixty minutes after test compound administration, animals were orally given a 2 ml suspension of 10% charcoal in 2.5% carboxymethylcellulose (kept at 37 °C±0.5). Fifteen minutes after the administration of charcoal, animals were sacrificed by cervical dislocation. Following midline laparotomy, the intestines were rapidly removed from the pylorus to the extremity of the cecum and spread out on a glass plate. The total length of the intestine and the distance covered by the charcoal were then measured immediately afterwards.

Salivary Secretion in Rats Salivary secretion was evaluated by measuring the weight gained by sublingual foam cubes after the subcutaneous administration of oxotremorine.[37] Compound **8** and AF-DX 116 were examined at doses of 15, 50, and 150 mg/kg by the oral route and administered to groups of 8 animals. Atropine (10 mg/kg *p.o.*) was used as a positive control drug. Male Sprague–Dawley rats (200—300 g) were randomly allocated to test treatments. Fifty minutes after test compound or vehicle administration, rats were injected subcutaneously with oxotremorine (0.5 mg/kg). Ten minutes after oxotremorine administration, the mouth of the animals was opened with gentle pressure on a snare, allowing placement in the oral cavity of a pre-weighed, absorbent foam cube. Foam cubes were held for 10 s and immediately after re-weighed. The difference between the first and second weight represents the saliva secreted.

Pupil Diameter in Rats Pupil diameter was evaluated under constant bright light with a dissecting microscope.[39] Compound **8** and AF-DX 116 were examined at doses of 15, 50 and 150 mg/kg by the oral route and administered to groups of 8 animals. Atropine (0.3 mg/kg *p.o.*) was used as a positive control drug. Sprague–Dawley rats (200—250 g) were randomly allocated to test treatments. Pupil diameter was measured just before and 60 min after test compound or vehicle administration.

In all *in vivo* functional selectivity studies, statistical comparisons were carried out using one-way analysis of variance for a completed randomized block.

Analgesic Activity in Mice The modified hot-plate procedure described by O'Callaghan and Holtzman was adopted.[41—43] Male Swiss albino mice (20—30 g) were used. Animals were kept at 22±1 °C with a 12-h light-dark cycle, and allowed access to food and water *ad libitum.* Mice were placed on a stainless steel thermostat plate set at 50±0.1 °C. A plastic cylinder was used to confine the mice to the heated surface of the hot-plate. The licking latency was defined as the time elapsing from thermal exposure to the licking of the fore or hind paws. A cut-off time of 75 s was adopted for licking latency in order to avoid unethical suffering and injury of the animals. Mean baseline licking latency was determined with three measurements before test compound administration. Test compounds were administered by a subcutaneous route to groups of 12—17 animals. Compounds **8**, **9**, **38** and AF-DX 116 were evaluated at doses of 5 mg/kg. Oxotremorine (0.2 mg/kg s.c.) was used as a positive control drug. After test compound administration, licking latencies were measured at 15 min intervals for 150 min. Statistical comparisons were carried out using repeated analysis of variance measurements.

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