SYNTHESIS AND SELECTIVE M-CHOLINOBLOCKING ACTIVITY OF TETRAAMINES. PART. I

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Among numerous preparations possessing M-cholinoblocking properties, a special group is formed by poly(methylene tetraamines):

$$ArCH_2NR^1–A–NR^2–B–NR^2–A–NR^1–CH_2Ar,$$
$$\text{I}$$

where A = $(CH_2)_n$, $n = 4-8$; B = $(CH_2)_m$, $m = 4-12$; Ar = Ph, *o*-$MeOC_6H_4$, hetaryl, etc.; R^1, R^2 = H or Me (in various combinations) [1].

These compounds significantly differ by their structures from conventional cholinolytics representing modified (e.g., "loaded" by introducing additional bulky substituents) acetylcholine or its agonists. Nevertheless, tetraamines I are capable of effectively blocking the M-cholinoreactive systems. In most cases, their affinity toward the M_2 receptor subtype is higher compared to that with respect to the M_1 and M_3 subtypes [1].

In recent years, certain relationships have been established between the pharmacological properties and chemical structures of tetraamines I [1]. However, all the compounds studied in this respect had very similar structures, whereby the fragments separating nitrogen atoms are represented by polymethylene chains with only the chain lengths or terminal aromatic groups varying from one to another compound. It was therefore of interest to extend the scope of our search, in particular, by partly replacing methylene units with aromatic groups capable of significantly modifying the properties of final products. Because of an increase in both the rigidity of molecules and their ability to bind to the π-electron acceptors, the conformational properties of the new compounds were expected to differ significantly from those of their precursors. This must obviously affect the biological activity of these compounds, including the specificity of interaction with M-cholinoreceptors of various subtypes.

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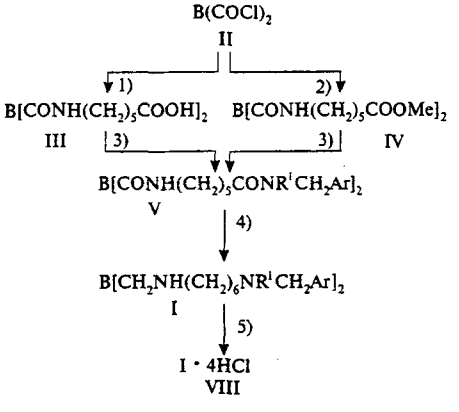
Proceeding from these considerations, we aimed at obtaining compounds in which a part of the methylene units in radical B (compound I) is replaced by phenylene residues. We also planned to study their biological properties. For the synthesis of various representatives containing unsubstituted nitrogen atoms, we have developed a sequence of reactions depicted in Scheme 1. As seen from this scheme, dichloroanhydride of dicarboxylic acid (II) produces acylation either of aminocaproic acid (Schotten – Baumann reaction) or its methyl ester hydrochloride in the presence of triethylamine in an organic solvent. The resulting diacid (III) or its dimethyl ester (IV) can be fused under similar conditions with an appropriate benzylamine to obtain tetraamide (V). Reduction of the latter compound with lithium aluminum hydride in diglyme leads to the target tetraamine I, from which a salt is obtained by conventional methods.

Scheme 1



Ar = Ph (a, c, e, g), 2-$MeOC_6H_4$ (b, d); B = 1,1-C_6H_4 (a – e), $(CH_2)_6$ (g); R^1 = H (a – d, g), Me (e).

1) $H_2N(CH_2)_5COOH$ (VI), NaOH, H_2O;

2) $H_2N(CH_2)_5COOMe \cdot HCl$ (VII), Et_3N, chloroform;

3) $ArCH_2NH_2$, smelting;

4) $LiAlH_4$, diglyme;

5) HCl, ethanol.

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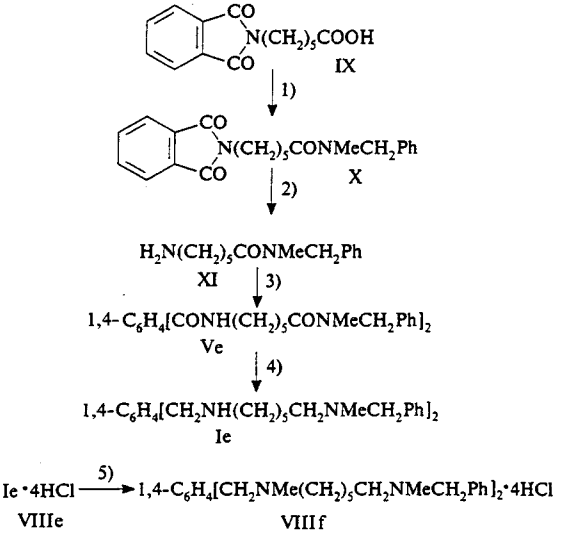
The method proposed in this work has important advantages over the procedures described for obtaining methoctramine and its close analogues [2, 3], in particular, because the reduction of amide to amine groups is performed only once (in the final stage) and the construction of the drug "skeleton" does not involve the formation of water-soluble amines, thus eliminating a number of difficulties.

Tetraamine I substituted at the peripheral nitrogen atoms was obtained according to Scheme 2. A key compound in this scheme was also the corresponding tetraamide (Vd) synthesized from terephthaloyl chloride and benzylmethylamide of aminocaproic acid (VII). The latter compound was obtained from the acid by the phthalimide method. Tetraamine with completely methylated nitrogen atoms (Ie) was synthesized by treating compound Ia with a mixture of formic acid and formalin.

Scheme 2



1a) $SOCl_2$, benzene;
1b) $PhCH_2NHMe$, Et_3N, benzene;
2) $H_2NNH_2 \cdot H_2O$, ethanol;
3) 1,4-$C_6H_4(COCl)_2$, Et_3N, acetonitrile;
4) $LiAlH_4$, diglyme;
5) HCOOH, CH_2O.

The methods outlined above were used to obtain six new tetraamines with various structures. Data on the structures and properties of these products are presented in Table 1. This approach was also used for the synthesis of methoctramine Ig.

EXPERIMENTAL CHEMICAL PART

The ^1H NMR spectra were measured on a Bruker AC-200 spectrometer. The spectra of tetraamine salts were obtained in D_2O or CH_3OD, tetraamides were dissolved in completely deuterated dimethyl sulfoxide or dimethylformamide, and tetraamine bases and other compounds were studied in $CDCl_3$. Characteristics of the ^1H NMR spectra of the products and intermediates are listed in Table 2.

The melting temperatures of the compounds studied were determined on a Boethius heating stage; the TLC analysis was performed of Silufol plates.

1,4-Bis(7-carboxy-3-azaheptanoyl)benzene (IIIa). To a solution of 2.03 g (10 mmole) of terephthalic acid dichloroanhydride in 20 ml of dry chloroform was added 2.62 g (20 mmole) of 6-aminocaproic acid. To this suspension was added dropwise with stirring 15 ml of a 20% aqueous KOH solution and the mixture was allowed to stand overnight at room temperature. Then the layers were separated, the aqueous phase was acidified, and the precipitated product filtered, dried, and recrystalized to obtain 2.74 g (71%) of compound IIIa; m.p., 218.5 – 220°C.

A similar procedure was used for the synthesis of diacid IIIb (see Tables 1 and 2).

1,4-Bis(7-methoxycarbonyl-3-azaheptanoyl)benzene (IVa). To a suspension of 2.03 g (10 mmole) of terephthalic acid dichloroanhydride and 3.64 g (20 mmole) of 6-aminocaproic acid methylate hydrochloride in 20 ml of dry acetonitrile was added this suspension was added dropwise with stirring 6 ml triethylamine. The deposit was initially almost completely dissolved, but in a few minutes precipitated again. Then the reaction mass was stirred for 2 h at room temperature and diluted with water. The precipitate was filtered, washed with 0.4 N HCl and water, dried, and recrystallized from chloroform to obtain 2.02 g (48%) of compound IVa; m.p., 161 – 162°C.

A similar procedure was used for the synthesis of compound IVb (see Tables 1 and 2).

1,4-Bis(7-benzylaminocarbonyl-2-azaheptanoyl)benzene (Va).

a) A mixture of diester IVa (3.0 g, 72 mmole) and benzylamine 2.46 g (23 mmole, 2.5 ml) was heated at 200 – 210°C for 2 h in a flask with reflux. The reaction mass initially melts and in a few minutes crystallizes. After the exposure, the smelt was dissolved in ethanol and diluted with water. The precipitate was filtered, washed with water, and dried to obtain 3.73 g (90%) of compound Va; m.p., 223 – 225°C. Tetraamide recrystallized from dimethylformamide melts at 233 – 235°C.

b) Analogous smelting of acid IIIa with a small excess of benzylamine yields 89% of tetraamide Va having the same characteristics as above.

A similar procedure was used for the synthesis of compounds Vb – Vg (see Tables 1 and 2).

1,4-Bis(10-phenyl-2,9-diaza-1-decyl)benzene (Ia). Tetraamide Va (5.5 g, 9.6 mmole) was added in a single portion to a suspension of 6.5 g (170 mmole) of lithium aluminum hydride in dry diglyme. The reaction mass was stirred, carefully heated to 70°C, held at this temperature for 5 h, and cooled. To the cooled mixture was added dropwise 2 – 3 ml of ethanol and 20 ml of 20% NaOH solution. Then diglyme was decanted from the slime, and the slime was doubly thoroughly washed with ether. The combined organic phases were dried and the solvent was removed to yield 5.57 g of a residue slightly wetted with diglyme. Crystallization from

acetone yields 2.86 g (56%) of a substance with m.p., 61 – 62°C. Additional crystallization increases the melting temperature to 65 – 66°C. The base isolated from purified hydrochloride melts at 71 – 72°C.

A similar procedure was used for the synthesis of other diamines I (see Tables 1 and 2).

Benzylmethylamide of phthalimidocaproic acid (X) was obtained from its chloroanhydride (synthesized by reaction of the corresponding acid IX [4] with excess thionyl chloride in benzene), an equivalent amount of benzylmethylamine, and a small excess of triethylamine in benzene; an oily product is obtained with a yield of 90%; ^{1}H NMR spectrum (δ, ppm): 7.90 – 7.60 (m, 4H, C_6H_4), 7.40 – 7.10 (m, 5H, C_6H_5), 4.55 (d, 2H, CH_2), 3.67 (m, 2H, NCH_2), 2.90 (d, 3H, CH_3), 2.46 (t, 2H, $COCH_2$), 1.85 – 1.30 (m, 6H, CH_2).

Benzylmethylamide of aminocaproic acid (XI) was obtained from the above phthalimide derivative X by a conventional method of decomposition with hydrazine hydrate in ethanol. Yield, 33.5%; b.p., 187°C (3 Torr); n_D^{20}, 1.5320; ^{1}H NMR spectrum (δ, ppm): 7.40 – 7.20 (m, 5H, C_6H_5), 4.65 (d, 2H, CH_2), 2.91 (d, 3H, CH_3), 2.68 (m, 2H, NCH_2), 2.38 (t, 2H, $COCH_3$), 1.80 – 1.30 (m, 6H, CH_2).

1,4-Bis(9-methyl-10-phenyl-1,8-dioxo-2,9-diaza-1-decyl)benzene (Ve) was obtained similarly to tetraamide Va, proceeding from terephthaloylchloride and amide XI.

1,4-Bis(9-methyl-10-phenyl-2,9-diaza-1-decyl)benzene (Id) was obtained by reducing tetraamide Vd according to the method used for the synthesis of tetraamine Ia.

1,4-Bis(2,9-dimethyl-10-phenyl-2,9-diaza-1-decyl)benzene (If). Tetrahydrochloride VIIIe (1 g, 1.5 mmole) was boiled for 3 h in a mixture of 8 ml formic acid and 4 ml formalin. Then the reaction mixture was evaporated to dryness in vacuum and crystallized from ethanol with ether. Yield of tetrahydrochloride VIIIe, 0.93 g (87%). The base of Ie was obtained by alkalinization of the salt. The properties of compound If are listed in Tables 1 and 2.

EXPERIMENTAL BIOLOGICAL PART

The selectivity profile of the synthesized compounds with respect to M-cholinoreceptors *in vitro* was determined by the method of radioligand analysis [5]. The test tissues were prepared from homogenates of the rat cerebral hemispheres (M_1 subtype), myocardium (M_2), and salivary glands (M_3).

The radioligand was represented by [3H] quinuclidinyl benzylate (Amersham) with a specific activity of 37 Ci / mole. The degree of nonspecific Binding did not exceed 15% of the total level; the counting efficiency was 40%.

We have also studied the interaction of compounds Ia and Ie with M-cholinoreceptors of right auricles (subtype M_2) and longitudinal small intestine muscles (M_3) of rats [6].

The reference preparation was methoctramine, known as one of the most selective M_2-cholinoblocking agents.

Acute toxicity of the synthesized compounds upon intraperitoneal injection was determined with respect to mice.

RESULTS AND DISCUSSION

As seen from the data presented in Table 3, most of the synthesized tetraamines exceed methoctramine [1] with respect to specificity of the M_2-cholinoblocking activity. Note that substituting methyl residues (compound VIIIe) for hydrogen at the terminal nitrogen atoms leads to some decrease in the selectivity at the expense of increasing affinity toward the M_1 and M_3 receptors, while the binding to the M_2 subtype remains unchanged. Methylation of all nitrogen atoms (compound VIIIf) results in a significant increase in the specificity

TABLE 1. Physicochemical Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C (solvent)	Empirical formula
Ia	56	65 – 66 (acetone)	$C_{34}H_{50}N_4$
Ia · 4HCl (VIIIa)		> 350 (ethanol – water, ~ 1 : 1)	$C_{34}H_{54}Cl_4N_4$
Ib	100	***	$C_{36}H_{54}N_4O_2$
Ib · 4HCl · $2H_2O$ (VIIIb)		250 – 252 (ethanol)	$C_{36}H_{62}Cl_4N_4O_4$
Ic	59	***	$C_{34}H_{50}N_4$
Ic · 4HCl · $4H_2O$ (VIIIc)		228 – 230 (ethanol – ester)	$C_{34}H_{62}Cl_4N_4O_4$
Id	27	***	$C_{36}H_{54}N_4O_2$
Id · $4C_2H_2O_4$		187 – 190 (aqueous methanol)	$C_{44}H_{62}N_4O_{18}$
Ie	52	126 – 127 (acetonitrile)	$C_{36}H_{54}N_4$
If · 4HCl · $2H_2O$		234 – 236 (isopropanol – methanol, ~ 1 : 1)	$C_{36}H_{62}Cl_4N_4O_2$
If		220 – 222 (ethanol – ester, ~1 : 1)	$C_{38}H_{58}N_4$
If ≤ 4HCl (VIIIf)	87	249 – 250 (ethanol – ester, ~1 : 1)	$C_{38}H_{62}Cl_4N_4$
Ig ≤ 4HCl (VIIIg)	18	180 – 195 ** (isopropanol – methanol, ~ 1 : 1)	$C_{36}H_{66}Cl_4N_4O_2$
IIIa	71	218 – 220 (dimethylformamide)	$C_{20}H_{28}N_2O_6$
IIIb	71	143 – 146 (aqueous ethanol)	$C_{20}H_{28}N_2O_6$
IVa	48	161 – 162(chloroform)	$C_{22}H_{32}N_2O_6$
IVb	88	69 – 72 (dioxane – ether, ~1 : 1)	$C_{22}H_{32}N_2O_6$
IVg	56	99 – 100 (ethanol)	$C_{22}H_{40}N_2O_6$
Va	90 (89)*	233 – 235 (dimethylformamide)	$C_{34}H_{42}N_4O_4$
Vb	67 (75)	130 – 131 (methyl ethyl ketone)	$C_{36}H_{46}N_4O_6$
Vc	70 (72)	169 – 170 (ethanol)	$C_{34}H_{42}N_4O_4$
Vd	(73)	139 – 142 (acetonitrile)	$C_{36}H_{46}N_4O_6$
Ve	91	108 – 110 (acetone)	$C_{36}H_{46}N_4O_4$
Vg	77	95 – 97 (ethanol)	$C_{36}H_{54}N_4O_6$

* Data ion parentheses refer to the yield from the corresponding diacid III.
** Reported m.p., 220°C [3].
*** Not crystallized.

TABLE 2. Parameters of ^1H NMR Spectra of the Synthesized Compounds[1)]

$$[Ar\underset{a}{-CH_2-}NH\underset{b}{-CH_2-}\underset{c}{(CH_2)_4}\underset{d}{-CH_2-}\underset{e}{NH}\underset{f}{-CH_2-}\underset{g}{]_2B} \quad I$$

Compound	a	b	c	d	e	f	g
Ia ≤ 4HCl	7.50	4.23	3.08	1.80 – 1.30	3.08	4.27	7.50
Ib ≤ 4HCl ≤ $2H_2O$	7.50 – 6.90, 3.90	4.22	3.01	1.95 – 1.30	3.01	4.27	7.57
Ic ≤ 4HCl ≤ $2H_2O$	7.50 – 7.40	4.23	3.00	1.65 – 1.35	3.00	4.80	7.50 – 7.40
Id ≤ $4C_2H_2O_4$	7.50 – 6.85, 3.80	4.15	2.97	1.61 – 1.38	2.97	4.17	7.50 – 6.85
Ie [2)]	8.07 – 7.30	3.50	3.41	1.70 – 1.30	2.40	3.50	8.07 – 7.30
If [3)]	7.40 – 7.20	3.50	2.38	1.50 – 1.25	2.38	3.48	7.40 – 7.20
Ig ≤ 4HCl	7.50 – 7.10, 3.90	4.25	3.05	1.70 – 1.35	3.05	3.05	1.70 – 1.35

$$[Ar\underset{a}{-CH_2-}NH\underset{b}{-CO-}\underset{c}{CH_2}\underset{d}{-(CH_2)_3-}\underset{e}{CH_2}\underset{f}{-NH}\underset{g}{-CO-}\underset{h}{]_2B} \quad V$$

Compound	a	b	c	d	e	f	g	h
Va	6.85 – 7.30	4.40	7.90	2.30	1.80 – 1.25	3.43	8.46	7.87
Vb	7.40 – 6.90, 3.90	4.41	8.12	2.33	1.80 – 1.40	3.45	8.68	7.40 – 6.90
Vc	7.35, 8.08	4.41	7.62	2.30	1.75 – 1.35	3.42	8.30	7.35, 8.08
Vd	7.30 – 6.80, 3.78	4.20	7.55	2.18	1.70 – 1.20	3.27	8.20	7.30 – 6.80
Ve [4)]	7.90 – 7.10	4.51		2.35	1.75 – 1.20	3.40	7.60	7.90 – 7.10
Vg	7.40 – 6.90, 3.70	2.35	7.80	2.15	1.70 – 1.20	3.20	4.48	1.70 – 1.20

$$[R-OCO\underset{a}{-CH_2-}\underset{b}{(CH_2)_3}\underset{c}{-CH_2}\underset{d}{-NH}\underset{e}{-CO}\underset{f}{]_2B}$$

III (R = H), IV (R = Me)

Compound	a	b	c	d	e	f
IIIa	–	2.29	1.70 – 1.30	3.25	8.60	7.90
IIIb	–	2.35	1.80 – 1.30	3.41	7.85	7.60
IVa	3.62	2.30	1.70 – 1.30	3.43	6.91	7.75
IVb	3.59	2.24	1.70 – 1.30	3.32	7.40	7.39
IVg	3.70	2.35	1.70 – 1.20	3.20	7.80	2.15[5)], 1.70 – 1.20[6)]

Notes. [1)] Protons of substituents in aromatic cycles, benzyl carbon atoms, and methyl ester residues give narrow singlets; amide NH groups give broad singlets; signals from protons of the NH groups of amines I and their salts VIII were not observed; nor were manifested protons of the carboxy groups; protons of the carbon atoms (non-benzyl) adjacent to the nitrogen atoms or CO groups give rise to triplets, sometimes poorly resolved; other protons give complex multiplets. [2)] Terminal nitrogen atoms replaced by methyl residues with the proton signals at 2.15 ppm. [3)] Nitrogen atoms replaced by methyl groups with a chemical shift of 2.19 ppm. [4)] Terminal nitrogen atoms replaced by methyl residues with a chemical shift of 2.90 ppm. [5)] CH_2CO absorption. [6)] CH_2 absorption.

TABLE 3. Acute Toxicity and Affinity of Tetraamines to M-Cholinoreceptors of Various Subtypes

Compound	K_i, [μM]			Selectivity profile			K_d, [μM]		Selectivity profile	LD_{50}, mg/kg
	M_1 (cerebral hemispheres)	M_2 (myocardium)	M_3 (salivary glands)	$\frac{M_1}{M_2}$	$\frac{M_3}{M_2}$	$\frac{M_3}{M_1}$	M_2 (auricles)	M_3 (auricles)	$\frac{M_3}{M_2}$	
VIIIa	1.4	0.17	16	8	94	11	0.6 ± 1	3.0 ± 0.4	5[1)]	39.8 ± 3.1
VIIIb	0.46	0.13	6.7	4	52	15	–	–		8.2 ± 1.2
VIIIc	0.94	0.15	7.7	6	50	8	–	–		35.7 ± 2.2
VIIIe	0.66	0.16	5.4	4	34	8	0.18 ± 0.03	1.3 ± 0.2	7.2 [2)]	35 ± 10
VIIIf	0.27	0.082	4.4	3	54	16	–	–		35 ± 10
VIIIg [3)]	0.186	0.035	1.3	5	37	7	–	–		21.3 ± 1.5

Notes. [1)] For a series of 10 experiments. [2)] For a series of 5 experiments. [3)] Methoctramine, $M_3/M_2 = 43$ [2].

due to an increase in the affinity of compound to the M_2 receptor. On the contrary, introducing methoxy groups into the o-position of phenyl residues (compound VIIIb) leads to some growth in the affinity with respect to all three subtypes of the cholinoreactive systems studied. However, the binding to M_2 cholinoreceptors increases to the lowest extent, which accounts for some reduction in the specificity of interaction. Transition from 1,4-to 1,3-phenylene group at the center of the molecule (compound VIIIc) also increases the affinity toward M_2 but to a lower degree compared to that for M_1 and M_3 subtypes, thus also leading to certain reduction in the specificity of tetraamine.

Study of the selectivity of compounds VIIIa and VIIIe on the samples of isolated organs (Table 1) also demonstrated a higher affinity of these compounds toward M_2 receptors as compared to their affinity to the M_3 subtype, but the effect was less pronounced than that observed in the biochemical experiments. Here, tetraamine VIIIe exhibited both greater activity and a somewhat higher selectivity compared to those of compound VIIIa.

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