SYNTHESIS AND BIOLOGICAL ACTIVITY OF 7,8-SUBSTITUTED THEOPHYLLINES

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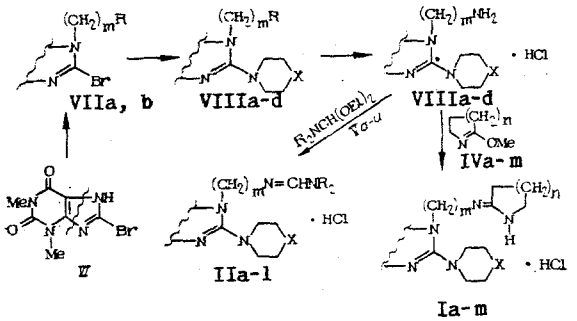
Theophylline and its derivatives such as Euphylline, Diprophylline, Proxyphylline, Bamifylline, and others, have been used for the arrest and prevention of bronchial asthma seizures. Besides acting as bronchodilators, they exhibit antiallergic properties by suppressing the release of anaphylactic reaction mediators from the mast cells and lymphocytes [6]. However, there is little difference between the effective therapeutic doses and those that cause side effects in these drugs. Thus, theophylline has a pronounced therapeutic effect in humans at a blood plasma concentration of 10-20 μg/ml, but undesirable side effects are manifested at a concentration of 15 μg/ml [5, 7]. Therefore, there is an urgent need to find new analogs of theophylline that have a high degree of bronchodilator and anti-allergic activity and are less toxic.

We synthesized 24 original 7,8-substituted theophyllines (Ia-m, IIa-i, and IIIa-d) and compared their pharmacological properties to those of theophylline with respect to bronchodilation (antihistamine activity), antianaphylactic action, and toxicity.

The I compounds were obtained by reacting the hydrochlorides of 7-(β-aminoethyl) (IIIa, c, m = 2)- and 7-(γ-aminopropyl) (IIIb, d, m = 3)-8-heterylтheophyllines with lactime esters (IV, n = 1-3). The II compounds were obtained by reacting III with acid amide acetals (V).

The starting hydrochlorides of compounds III were synthesized from 8-bromotheophylline (VI) [2] by reacting the latter with ω-alkylhalidephthalamides in DMFA in the presence of potash, and the replacement of the bromine in the resultant compounds (VII) by a morpholine or piperidine residue followed by the cleavage of the N-substituted phthalimides (VIII) by hydrazine hydrate [3] in boiling butanol. HCl was then used to separate the target hydrochlorides.



X = CH_2 (Ia-f IIa-d; IIIa, b; VIIIa, b); O (Ih-m Ie-i, IIIc, d; VIIIc, d); R-Me (IIa c, e h; (Va, c, e, h); $O(CH_2CH_2)_2$ (IIb, d, f, i; (Vb, d, f, i); pathalimido (VII, VIII); m = 2 (Ia-c, h-j; IIa, b, e, f; III a, b, VIIa; VIIIa, c); 3 (I d-f, k-m, IIc, d, e, i, IIIb, d; VIIb, VIIIb, d); p = 1 (Ia, d, h, k; IVa, h, h, k); 2 (Ib, e, i, l; IVb, e, i, l); 3 (Ic, f, j, o, IVc, f, j, o).

EXPERIMENTAL CHEMICAL

IR spectra were recorded on a Perkin-Elmer 457 instrument (Sweden) in the form of a paste with petroleum jelly. UV spectra were recorded on a Hitachi (Japan) EPS-3T spectrophotometer in ethanol. Mass spectra were recorded on a MAT-112 instrument (ionization potential 50 eR, temperature of ionization chamber 140°C). TLC performed on Silufol UV-254

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TABLE 1. Physiochemical Properties of the Synthesized Compounds

Compound	mp, °C	Yield, %	Found, %				Empirical formula	Calculated, %				IR spectra, max, cm^{-1}
			C	H	Cl	N		C	H	Cl	N	C=O, C=O
Ia	224—6	61,9	52,55	6,87	8,65	24,43	$C_{18}H_{27}N_7O_2 \cdot HCl$	52,80	6,89	8,66	23,95	1690, 1648, 1500
Ib	226—8	50,1	53,80	7,13	8,58	23,33	$C_{19}H_{29}N_7O_2 \cdot HCl$	53,83	7,08	8,38	23,14	1710, 1650, 1510
Ic	234—5	64,2	54,46	7,49	8,26	22,43	$C_{20}H_{31}N_7O_2 \cdot HCl$	54,85	7,36	8,10	22,39	1690, 1643, 1515
Id	220—21	50,2	53,86	7,21	8,27	22,36	$C_{19}H_{29}N_7O_2 \cdot HCl$	53,84	7,08	8,38	23,14	1700, 1660, 1513
Ie	134—6	67,1	53,32	7,40	8,04	21,98	$C_{20}H_{31}N_7O_2 \cdot HCl$	53,75	7,39	7,95	21,95	1690, 1650, 1515
If	202—4	54,4	55,86	7,62	7,89	22,05	$C_{21}H_{33}N_7O_2 \cdot HCl$	55,80	7,58	7,89	21,69	1700, 1658, 1507
Ih	217—8	68,6	49,50	6,46	8,54	23,81	$C_{17}H_{25}N_7O_3 \cdot HCl$	49,57	6,36	8,61	23,81	1690, 1648, 1508
Ii	155—6	64,5	48,61	6,80	7,77	22,20	$C_{18}H_{27}N_7O_3 \cdot HCl$	48,70	6,76	7,99	22,09	1690, 1642, 1508
Ij	232—4	79,9	51,18	6,70	8,16	22,19	$C_{19}H_{29}N_7O_3 \cdot HCl$	51,88	6,83	8,08	22,30	1705, 1654, 1512
Ik	207—8	67,2	50,71	6,77	8,42	23,39	$C_{18}H_{22}N_7O_2 \cdot HCl$	50,78	6,57	8,33	23,04	1705, 1670, 1505
Il	218—9	65,1	51,68	6,78	8,17	22,16	$C_{19}H_{29}N_7O_3 \cdot HCl$	51,88	6,83	8,08	22,30	1705, 1670, 1500
Im	234—6	83,8	52,63	7,41	7,73	21,98	$C_{20}H_{31}N_7O_3 \cdot HCl$	52,94	7,65	7,81	21,60	1700, 1655, 1500
IIa	184—6	52,8	50,38	7,27	8,65	23,97	$C_{17}H_{27}N_7O_2 \cdot HCl$	50,18	7,13	8,73	24,11	1710, 1655, 1515
IIb	222—4	62,3	51,71	7,09	8,14	22,10	$C_{19}H_{29}N_7O_3 \cdot HCl$	51,87	6,82	8,06	22,29	1705, 1653, 1510
IIc	129—32	43,2	50,03	7,24	8,24	23,09	$C_{18}H_{29}N_7O_2 \cdot HCl$	50,29	7,45	8,26	22,82	1692, 1648, 1505
IId	214—6	43,3	52,67	6,95	8,05	21,39	$C_{20}H_{31}N_7O_3 \cdot HCl$	52, 92	7,06	7,83	21,62	1700, 1640, 1505
IIe	162—4	56,8	45,72	6,60	8,67	23,21	$C_{16}H_{25}N_7O_3 \cdot HCl$	45,98	6,75	8,49	23,46	1695, 1640, 1500
IIf	253—5	52,3	48,69	6,49	8,18	22,43	$C_{18}H_{27}N_7O_4 \cdot HCl$	48,92	6,39	8,02	22,19	1690, 1640, 1507
IIh	213—4	49,8	47,61	6,67	8,28	23,08	$C_{17}H_{27}N_7O_3 \cdot HCl$	47,27	6,49	6,23	22,71	1695, 1645, 1508
IIi	216—8	55,1	49,56	6,57	7,97	21,30	$C_{19}H_{29}N_7O_4 \cdot HCl$	50,06	6,58	7,78	21,51	1690, 1660, 1495
IIIa	277—9	66,7	49,01	6,89	10,24	25,06	$C_{14}H_{22}N_6O_2 \cdot HCl$	49,05	6,72	10,36	24,53	1690, 1663, 1495
IIIb	227—9	58,2	50,28	6,90	9,94	23,65	$C_{15}H_{24}N_6O_2 \cdot HCl$	50,51	7,00	9,94	23,57	1700, 1650, 1550
IIIc	262—4	46,4	44,88	6,05	9,94	24,39	$C_{13}H_{20}N_6O_3 \cdot HCl$	45,28	6,09	10,30	24,38	1710, 1650, 1550
IIId	250—52	71,2	46,81	6,59	9,63	23,43	$C_{14}H_{22}N_6O_3 \cdot HCl$	46,88	6,41	9,89	23,42	1703, 1656, 1540
VIIa	239—42	65,0	47,17	3,32	18,36*	16,61	$C_{17}H_{14}BrN_5O_4$	47,23	3,26	18,49*	16,20	1780, 1710, 1663, 1540
VIIb	196—7	80,9	48,64	3,52	17,89*	15,56	$C_{18}H_{16}BrN_5O_4$	48,43	3,62	17,91*	15,70	1700, 1655, 1530
VIIIa	215—7	53,4	60,41	5,67	—	19,51	$C_{22}H_{24}N_6O_4$	60,55	5,50	—	19,27	1770, 1720, 1645, 1520
VIIIb	197—8	76,7	61,38	5,88	—	18,39	$C_{23}H_{26}N_6O_4$	61,35	5,77	—	18,66	1770, 1705, 1640, 1505
VIIIc	210—11	83,0	57,63	5,05	—	19,41	$C_{21}H_{22}N_6O_5$	57,53	5,02	—	19,18	1770, 1718, 1660, 1510
VIIId	189—90	70,4	58,36	5,67	—	18,73	$C_{22}H_{24}N_6O_5$	58,42	5,31	—	18,58	1765, 1710, 1655, 1505

NOTE. Asterisk — Br. The UV spectra for the aqueous solutions of all compounds exhibited two absorption maxima at 210-212 and 290-300 nm, and one minimum at 260-262 nm. Compounds Ia, m, and IIb were crystallized from ethanol, Ib, d, e, IIa, c, d, and h were crystallized from a mixture of isopropanol and ethyl acetate, compounds Ic, f-k, IIe, f, i, IIIc-d, VIIa, b, and VIIIa, c from DMFA, IIIa from DMFA (aqueous), and IIIb, VIII b, d from a mixture of DMFA and ethanol. Compounds Ii, IIc, e, and h were crystallized in the form of hydrates, and Ie and IIa were crystallized in the form of hemihydrates.

plates (Czechoslovakia), development in UV light or iodine vapor. Analytical results of the synthesized compounds are given in Table 1.

7-β-[(Pyrrolidene-2)imino]ethyl-8-morpholinotheophylline HCl (Ih, m = 2, n = 1, X = O). A 5-g (14.5 mmole) portion of IIIc (m = 2, X = O) was boiled with 1.9 g (19 mmoles) of O-methylbutyrolactime (IVh, n = 1) in 20 ml of abs. ethyl alcohol for 2 h. The resultant solution was cooled and brought to pH 4 by the addition of an alcohol HCl solution and left in a refrigerator for 12 h. The resultant precipitate I (m = 2, n = 1, X = O) was filtered off and crystallized. The entire group of I compounds was obtained in the same way.

N-[β-(8-Morpholinotheophyllinyl-7)ethyl]-N^1,N^1-dimethylformamidine HCl (IIe, m = 2, X = O, RR = Me). A 2.3-g (6.67 mmoles) portion of IIIc (m = 2, X = O) was boiled with 1.5 g (10 mmoles) of diethylacetal dimethylformamide (Ve, RR = Me_2) in 10 ml of abs. ethanol for 2 h. The resultant solution was cooled and brought to pH 4 by the addition of an alcohol HCl solution and left in a refrigator for 12 h. The resultant precipitate IIe (m = 2, X = O, RR = Me_2) was filtered off and crystallized. The entire group of II compounds was obtained in a similar fashion.

7-(β-Aminoethyl)-8-morpholinotheophylline HCl (IIIc, m = 2, X = O). A mixture of 22.3 g (51 mmoles) of VIIIc (m = 2, X = O) and 5.5 g (110 mmoles) of hydrazine hydrate were boiled in 200 ml of butanol for 3 h and evaporated until dry. The residue was boiled with 100 ml of 1 N HCl and cooled. The phthalic hydrazide was filtered off and the filtrate was evaporated until dry and the residue was crystallized. The resultant product was IIIc (m = 2, X = O). The compounds IIId (m = 3, X = O) and IIIa, b (m = 2, 3, X = CH_2) were obtained in a similar fashion.

7-(β-Phthalimidoethyl)-8-bromotheophylline (VIIa, m = 2). A mixture containing 20.8 g (80 mmoles) of 8-bromotheophylline (VI), 11 g (80 mmoles) of calcined potash, and 24.3 g (96 mmoles) of β-bromoethylphthalimide was stirred in 200 ml of DMFA for 2 h at 120°C and then cooled. The resultant precipitate was filtered off and washed with water and alcohol. Compound VII (m = 3) was obtained in the same way.

7-(β-phthalimidoethyl)-8-morpholinotheophylline (VIIIc, m = 2, X = O). A mixture containing 8.7 g (20 mmoles) of VIIa (m = 2), 2.8 g (20 mmoles) of potash, and 2.2 g (25 mmoles) of morpholine was stirred in 30 ml of DMFA for 2 h at 120°C and filtered. After, the filtrate was cooled the resultant precipitate was VIIIc (m = 2, X = O). It was separated and washed with water and alcohol. Compounds VIIId (m = 3, X = O) and VIIa, b (m = 2, 3 X = CH_2) were obtained in a similar fashion.

EXPERIMENTAL—PHARMACOLOGICAL

The broncholytic (antihistamine) activity of the synthesized derivatives was studied on narcotized guinea pigs weighing 300-500 g, and was evaluated by the extent to which they diminished bronchial constriction induced by an intravenous injection of histamine dihydrochloride at a dose of 10 μg/kg. The tests were conducted in accordance with the Konzett-Rössler method as modified by M. É. Kaminka [1]. The substances were administered IV at doses that were 0.1 of the LD_{50} indicated in Table 2.

Antianaphylactic activity of the compounds was evaluated by the rear limb active anaphylaxis response (RLAR) in rats [4] as modified by us. The experiment was conducted on mongrel white rats of both sexes weighing 150-160 g. The animals were sensitized by a single 10-μg IP injection of ovalbumin (OA) and 100 mg of aluminum oxide hydrate in 0.5 ml of 0.9% NaCl. After 14 days a subplantar dose of 10 μg of OA in 0.1 ml of 0.9% NaCl was injected into the right rear limb. A 0.1-ml dose of 0.9% NaCl without OA was injected subplantarly into the left rear limb which served as the control. Edema was measured with a Ugo Basile (Italy) plethysmometer 30 min after the resolving dose. The reaction's intensity was judged by the percent of edema expressed as

$$\frac{A-B}{B} \cdot 100\%,$$

where A is the volume of the limb into which the resolving dose of OA was injected, and B is the control limb. The rats in which the edema percent was not less than 20 were considered to reaction-positive and were used for further study. In preliminary experiments we found that their average anti-OA IgE-antibody titer was 1/160. IgG for the 2a-antibody were lacking. Reaction-positive rats selected on the day before the experiment were used to determine the compounds' antianaphylactic activity. The resolving dose (25 μg of OA in 0.1

TABLE 2. The Effect of 7,8-Disubstituted Theophyllines on Histamine-Induced Bronchospasm in Narcotized Guinea Pigs and on Anaphylactic Rear Limb Edema in Rats, and the LD_{50} for White Mice

Compound	Bronchostatic reaction, % of the control	RLAR, % of the control	LD_{50}, mg/g (Iv)
IIIc	98±5	56±12*	720
IIIa	104±11	154±32	195
IIId	106±8	48±10*	430
IIIb	97±8	108±27	248
IIe	115±13	145±45	180
IIa	100±10	121±20	190
IIh	95±7	101±17	90
IIc	99±5	76±13	110
Ih	97±15	74±12	70
Ia	111±10	96±13	60
Ik	110±14	88±14	64
Id	108±11	95±4	44
Ii	108±9	51±6*	64
Ib	112±20	81±7	92
Il	92±5	72±24	65
Ie	107±16	87±7	61
IJ	106±10	28±6*	59
Ic	109±8	63±14	47
Im	93±9	108±21	60
If	98±12	89±11	32
IIf	97±6	110±27	285
IIb	101±2	77±7	280
IIi	97±7	91±17	175
IId	103±14	67±14	300
Theophylline	3±2*	31±6*	192

Note: * — $P < 0.05$ in comparison to the control

ml of 0.9% NaCl) was injected into the left limb, and the same volume of 0.9% NaCl was injected into the right limb. The test substances were administered IP in a dose of 50 mg/kg IP 30 min before the resolving dose of the antigen was injected. Antianaphylactic activity was judged by the extent to which the substances reduced the edematous reaction.

Acute toxicity was assayed on mice weighing 16-18 g. The test compounds were administered IV. LD_{50} was calculated by the Litchfield-Wilcoxon method.

The activity and toxicity of the compounds under study were compared to that of theophylline and the resultant data were statistically processed. The Student's criterion was used to establish the reliability of differences at $P = 0.05$.

The results presented in Table 2 indicate that none of the examined compounds prevented histamine-induced bronchoconstriction at doses of 0.1 LD_{50}. At the same time, some of those compounds significantly (by 30-50%) suppressed antigen-induced limb edema in sensitized rats, although their activity did not exceed that of theophylline at the same dosage. The most active and least toxic of the examined series were compounds IIIc, d (m = 2, X = O) which combined short aminoethyl or aminopropyl substituents in position 7 with a morpholine substituent in position 8. The substitution of morphiline by piperidine or the addition of dimethylformamide or morpholinomethyl fragments to the amino alkyl chain resulted in the disappearance of the antiedema action, whereas the introduction of 5-, 6-, and 7-member nitrogen-containing heterocycles in position 7 resulted in the compounds' greater toxicity.

Thus, among the examined, 7- and 8-substituted theophyllines we found compounds that were capable of suppressing anaphylactic edema but exhibited no antihistamine (broncholytic) activity. Inasmuch as the action of the injected histamine on the bronchial smooth musculature was identical to the pathophysiological stage of an immediate hypersensitivity reaction (according to A. D. Ado, 1978), one might assume that these compounds' suppression of RLAR is related to a blockage of allergy mediator release. This indicates that it might be possible to obtain new substances among the 7,8-substituted theophyllines that exhibit selective antianaphylactic activity and that have a low degree of toxicity.

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