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Preliminary communication

Synthesis, toxicological and pharmacological assessment of 7-substituted derivatives of 1,3-dimethylxanthine

PT Peikov[1], AB Zlatkov[1], MT Markov[2], ND Danchev[2], DI Ivanov[2], JT Panova[2]

[1]*Department of Pharmaceutical Chemistry;*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, 2 Dunav Street, Sofia 1000, Bulgaria*

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Summary — The synthesis of two 7-substituted theophylline derivatives from the theophylline sodium salt, dichloroalkane and *N*-methyl-*N*-cyclohexylamine is reported. The structures of the synthesized compounds were proved by microanalyses and ^{1}H-NMR data. Acute toxicity studies of the compounds were performed on mice and rats. A comparative pharmacological study of the *in vivo* broncholytic effect of the derivatives and aminophylline showed that the new compounds have different bronchodilatory activity and the compound 7-[2-(*N*-methyl-*N*-cyclohexyl)-aminoethyl]-1,3-dimethylxanthine tartrate **3a** was more active than 7-[3-(*N*-methyl-*N*-cyclohexyl)-aminopropyl]-1,3-dimethylxanthine hydrochloride **3b**. It was demonstrated that compound **3b** and aminophylline exerted the strongest inhibitory effect on the enzyme activity of phosphodiesterase in lung homogenate. Compound **3a** showed a slight inhibitory effect on the enzyme activity in this homogenate. The possible mechanisms of the broncholytic effects of these compounds are discussed.

1,3-dimethylxanthine / theophylline / aminophylline / acute toxicity / bronchodilatory activity / phosphodiesterase activity

Introduction

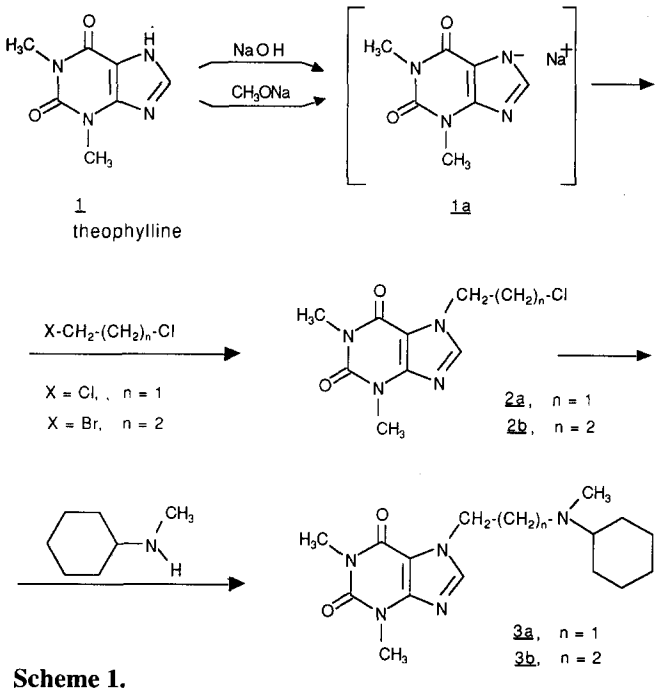
Theophylline and its derivatives possess bronchodilatory activity. The most widely used drug of this group is aminophylline, which is a soluble complex formed between theophylline and ethylenediamine [1]. However, this drug exhibits some side effects including severe allergic reations and cardiotoxicity [2]. It is possible to obtain new compounds with biological activity and toxicity comparable to that of aminophylline, but with lower or no cardiotoxic effects, by chemical modification of the theophylline structure.

The new methylxanthine derivative series has been examined for bronchodilatory activity and toxicity by the OASIS (Optimized Approach Based on Structure Indices Set) method [3]. This method may be considered to be an expanded and optimized variation of the Hansch physicochemical method [4].

We report here the preparation of 2 modified theophylline derivatives, and describe their pharmacological and toxicological effects.

Chemistry

Theophylline **1** was treated with an aqueous solution of sodium hydroxide or sodium methoxide in methanol at a molar ratio of 1:1 to produce the theophylline sodium salt **1a** (scheme 1). Treatment of **1a** with dihaloalkane at molar ratio 1:8 and 1:10.2 afforded 7-haloalkyltheophylline derivatives **2a** and **2b** in 62–90% yield. 7-Haloalkyltheophyllines (**2a, 2b**) then reacted with *N*-methyl-*N*-cyclohexylamine at a 1:4 molar ratio to produce 7-[2-(*N*-methyl-*N*-cyclohexyl)-aminoethyl]-1,3-dimethylxanthine **3a** and 7-[3(*N*-methyl-*N*-cyclohexyl)-aminopropyl]-1,3-dimethylxanthine **3b**. The reaction time from the appearance of **2a**



Scheme 1.

and **2b** until their exhaustion was monitored by TLC. In this case *N*-methyl-*N*-cyclohexylamine was used both as a reagent and an acceptor of the hydrochloride liberated. On completion of the process, unreacted amine was regenerated.

Pharmacology

Newly synthesized compounds **3a** and **3b** were investigated for acute toxicity, *in vivo* bronchodilatory activity and *in vitro* antagonism of acetylcholine-induced tracheal contraction. An assessment of the compounds' effect on phosphodiesterase activity in rat lung and heart homogenates was made. Aminophylline was used as a reference substance.

Results and discussion

Analysis of the obtained experimental data on the acute toxicity (LD_{50}) of **3a** and **3b**, as compared to aminophylline, showed that compounds **3a** and **3b** had acute toxicity (LD_{50}) close to the standard substance aminophylline (table I).

As shown by *in vivo* experiments (table II) on guinea pigs, the bronchodilatory effects of compound **3a** and aminophylline were optimal and continued the longest amount of time (> 30 min). The broncholytic effect of compound **3b** in equitoxic doses (1/5 and 1/10 parts of LD_{50} *via* iv administration) was statistically less significant than that of **3a** and aminophylline.

In vitro experiments on isolated guinea-pig trachea show that the bronchodilatory effect on acetylcholine-induced spasm exerted by compound **3a** (pD_2 = 4.54) is statistically more significant than that exerted by

Table I. Acute toxicity (LD_{50})* of compounds **3a, 3b** and aminophylline.

Compound	*Animal*	*Administration route*	*LD_{50}(mg/kg) (range of values)*
	mouse	ip	206.9 (162.7 – 262.9)
	mouse	*op*	598.1 (495.2 – 722.4)
3a	rat	iv	116.5 (87.9 – 154.3)
	rat	ip	206.9 (162.7 – 262.9)
	rat	*op*	2880.0 (2293.0 – 3618.0)
	mouse	ip	116.5 (87.9 – 154.3)
	mouse	*op*	299.1 (229.0 – 390.6)
3b	rat	iv	102.5 (72.6 – 144.9)
	rat	ip	116.5 (87.9 – 154.3)
	rat	*op*	1639.0 (1331.1 – 2018.3)
	mouse	iv	125.0 (111.2 – 141.1)
	mouse	ip	180.1 (158.6 – 205.3)
aminophylline	mouse	*op*	410.0 (328.9 – 513.7)
	rat	iv	103.8 (76.1 – 129.4)
	rat	ip	129.1 (114.3 – 146.9)
	rat	*op*	1120.6 (691.8 – 1582.1)

* The data were calculated by Litchfield–Wilcoxon method [5].

Table II. Bronchodilating activity (*in vivo*) of **3a, 3b** and aminophylline on the guinea pig.

Compound	*Doses (mg/kg) iv*	*Decrease of acetylcholine (10 mg/kg)-induced bronchospasm in the guinea pig (%)*			
		2 min	*10 min*	*20 min*	*30 min*
	6	53.8 ± 5.4	34.6 ± 4.3	–	–
3a	12	65.6 ± 5.9	51.6 ± 6.5	27.2 ± 2.9	–
	24	92.0 ± 5.8	69.2 ± 6.3	44.5 ± 3.7	23.2 ± 2.5
	5	22.7 ± 3.2	–	–	–
3b	10	36.0 ± 4.1*	19.1 ± 1.9*	–	–
	20	67.8 ± 5.7*	52.0 ± 5.6*	24.6 ± 3.1*	–
aminophylline	12	80.1 ± 7.2	64.3 ± 7.8	40.0 ± 4.7	–
	24	89.6 ± 8.1	70.8 ± 6.9	45.8 ± 5.1	24.6 ± 2.8

The data were presented in M ± SD (n = 6); * $p \leq 0.05$ statistically significant difference in comparison with aminophylline and **3a**.

aminophylline (pD_2 = 4.15) (table III). Compound **3b** does not influence the acetylcholine spasm in guinea-pig trachea and relaxation was observed only in 3 x 10^{-4} mol/l concentration.

Table III. Influence of **3a** and **3b** and aminophylline on acetylcholine-induced guinea-pig trachea contraction *in vitro*.

Compound	*Concentration (mol/l)*	*Relaxation (%)*
	1 x 10^{-6}	0
	3 x 10^{-6}	6.1 ± 2.6
3a[a]	1 x 10^{-5}	27.7 ± 7.8*
	3 x 10^{-5}	61.8 ± 7.7*
	1 x 10^{-4}	100.0 ± 0*
	3 x 10^{-4}	-
	1 x 10^{-6}	0
	3 x 10^{-6}	0
3b	1 x 10^{-5}	0
	3 x 10^{-5}	0
	1 x 10^{-4}	6.9 ± 7.3
	3 x 10^{-4}	31.3 ± 6.2
	1 x 10^{-6}	0
	3 x 10^{-6}	4.8 ± 1.8
aminophylline[b]	1 x 10^{-5}	12.2 ± 3.4
	3 x 10^{-5}	37.2 ± 7.0
	1 x 10^{-4}	63.3 ± 6.4
	3 x 10^{-4}	100 ± 0

The data were presented in M ± SD (n = 6); *$p \leq 0.05$ statistically significant difference in comparison with aminophylline. [a]**3a**: EC_{50} = 2.9 x 10^{-5} mol/l, r = 0.962, pD_2 = 4.54; [b]aminophylline: EC_{50} = 7.1 x 10^{-5} mol/l, r = 0.958, pD_2 = 4.15.

The effect on phosphodiesterase activity in rat lung and heart homogenates of the compounds **3a** and **3b** was comparatively studied with aminophylline at concentrations 2 x 10^{-3} M and 2 x 10^{-4} M. It was demonstrated that in lung and heart homogenates compound **3b** and aminophylline exerted a stronger inhibitory effect on the enzymic activity of phosphodiesterase in concentration 2 x 10^{-3} M than **3a** ($p \leq 0.05$, table IV).

The results of the *in vivo* and *in vitro* pharmacological screening experiments on guinea-pig avascular (bronchial) smooth muscles show that compound **3a** and aminophylline have a remarkable bronchodilation effect. Compound **3a** has a significantly higher bronchodilating activity *in vitro* (table III) than aminophylline in concentrations 1 x 10^{-5}, 3 x 10^{-5} and 1 x 10^{-4} mol/l and similarly *in vivo* (table IV). The difference between *in vivo* and *in vitro* experiments may be due to different biological availability.

It is well established that one of the mechanisms of bronchodilatory action of theophylline derivatives is related to their effect on the level of cyclic adenosine 3', 5'-monophosphate in the cells of bronchial smooth muscles, compared to their effect on phosphodiesterase concentration [6, 7]. Pharmacobiochemical studies into the enzymic activity show that compound **3a** at a concentration of 2 x 10^{-4} M does not exert an inhibitory effect on phosphodiesterase in lung and heart homogenates of rat.

The absence of phosphodiesterase inhibition in heart homogenates of rats is an advantage of **3a**, because it shows no cardiotoxic effects.

The fact that **3a** induces bronchodilation without any significant inhibition of lung phosphodiesterases, suggests the presence of some other mechanism of action. Other pharmacodynamic mechanisms that may

Table IV. Influence of **3a, 3b** and aminophylline on the phosphodiesterase activity in rat lung and heart.

Compound	*Concentration (M)*	*Phosphodiesterase activity lung (%)*	*heart (%)*
3a	2×10^{-3}	88.3 ± 4.3*	94.2 ± 5.8*
	2×10^{-4}	[100.0]	[100.0]
3b	2×10^{-3}	69.3 ± 4.5	63.1 ± 5.7
	2×10^{-4}	94.7 ± 5.5	97.5 ± 4.6
aminophylline	2×10^{-3}	63.2 ± 2.1	63.2 ± 4.5
	2×10^{-4}	93.4 ± 4.4	93.3 ± 5.3

The data were presented in M ± SD in % ($n = 6$); *$p \leq 0.05$ statistically significant difference in comparison with aminophylline.

be considered are mechanisms of methylxanthine action such as Ca^{2+} influx and cell distribution, prostaglandin antagonism [8], and the influence on the tracheobronchial purinergic receptors [9].

The studies of Sakai *et al* [10] and Mijamoto *et al* [11] suggest that both cyclic adenosine monophosphate-phosphodiesterase inhibitory activity and adenosine (A_1) antagonistic action are important in the bronchodilatory effect of 1-, 3- and 7-substituted xanthine derivatives. These authors found relationships between *in vitro* tracheal relaxant activities of similar compounds, their activities for adenosine (A_1) receptors in the brain membrane, and their inhibition of cyclic adenosine monophosphate-phosphodiesterase in the tracheal muscle.

Conclusion

Comparative pharmacological and toxicological experiments on the compounds **3a** and **3b** indicated that **3a** has a higher broncholytic effect *in vitro* and the same *in vivo* effect. The acute toxicity (LD_{50}) of **3a** is close to that of the preparation aminophylline. The broncholytic effect of **3b** *in vitro* and *in vivo* is less than aminophylline activity.

The cost and time required for the experiments were significantly decreased by a prediction of the biological activity of modified theophylline derivatives **3a** and **3b** (selected from a series of about 30 structures) by means of the OASIS method.

Experimental protocols

Chemistry

Melting points were measured in °C and were corrected. ^{1}H-NMR spectra were recorded at ambient temperature on a Bruker 250 WM (250 MHz) spectrometer in DMSO-d_6 and D_2O. Chemical shifts are given in ppm (δ) relative to TMS as an internal standard. IR spectra (KBr) were recorded on a IR 20 (Karl Zeiss, Jena) spectrophotometer. TLC was performed on Merck 60 F_{254} (0.20 mm) sheets with acetone/ethanol/chloroform/formic acid solvent (3:3:4:1) and detected at UV 254 nm. The novel structures were supported by microanalyses (Microanalytical Unit, Faculty of Pharmacy, Sofia) and the characteristic IR and NMR data quoted.

7-(2-Choroethyl)-1,3-dimethylxanthine **2a**

Theophylline **1** (150.0 mmol, 29.70 g) was added to a solution of sodium hydroxide (150.0 mmol, 6.0 g) in 200 ml water. 1,2-Dichloroethane (1.20 mol, 118.66 g) in 300 ml *i*-propanol was then added with stirring. The mixture was heated under reflux at 78–80°C for 76.5 h. After filtration and removal of the solvent, the residual solid was extracted with chloroform (3 x 200 ml). Combined chloroform extracts was dried over anhydrous sodium sulfate and after filtration the solvent was removed. The product **2a** crystallized from ethanol in 90% yield, mp: 120–122°C [12].

7-(3-Chloropropyl)-1,3-dimethylxanthine **2b**

Sodium methoxide (100.0 mmol, 18.0 g 30% methanol solution) was added to a solution of theophylline **1** (100.0 mol, 18.0 g) in 600 ml methanol. The mixture was heated under reflux until dissolution of **1**. 1-Bromo-3-chloropropane (1.02 mol, 160 g) was then added. After refluxing for 17 h the solvent was removed. The residue was dissolved in chloroform and filtered. The filtrate was then evaporated and crude **2b** crystallized out from methanol in 62% yield, mp 151-152°C [13].

7-[2-(N-Methyl-N-cyclohexyl)-aminoethyl]-1,3-dimethylxanthine tartrate **3a**

A mixture of 7-(2-chloroethyl)-1,3-dimethylxanthine **2a** (2.4 g, 10.0 mmol) and *N*-methyl-*N*-cyclohexylamine (4.5 g, 40.0 mmol) was heated at 100°C for 285 min. After cooling to 50°C, the residual oil was dissolved in 10 ml absolute EtOH treatment with 20% of an alkaline solution of tartaric acid to pH 6–6.5 and recrystallization from absolute EtOH gave 2.6 g (55%) of **3a**, mp 181.5–183°C; (found: C, 51.20; H, 6.08; N, 14.49; calc $C_{20}H_{31}N_5O_8$ requires C, 51.16; H, 6.66; N, 14.92%). IR, cm^{-1}: 1700, 1680, 1665, 1550. ^{1}H-NMR: 8.04 s (1H, C^8–H); 4.33 t (2H, $J = 4.1$ Hz, –N^7–CH_2–); 4.24 s (2H, 2 x CH from tartaric acid); 3.42 s (3H, N–CH_3); 3.23 s (3H, N–CH_3); 2.87 t (2H, $J = 6.3$ Hz, –CH_2–N); 2.52–2.39 m (1H, –N–CH–, partially covered by DMSO-d_6 signal); 2.31 s (3H, N–CH_3); 1.66, 1.02 m (10H, cyclohexyl).

7-[-3-(N-Methyl-N-cyclohexyl)-aminopropyl]-1,3-dimethylxanthine hydrochloride **3b**

Compound **3b** was prepared from 7-(3-chloropropyl)-1,3-dimethylxanthine (2.6 g, 10.0 mmol) and *N*-methyl-*N*-cyclohexylamine (4.5 g, 40.0 mmol) as described above for 150 min. The residual oil was dissolved in 10 ml warm absolute EtOH after cooling to 50°C. To the resulting solution a saturated HCl/EtOH was added dropwise (pH = 5.5–6). After evaporating the solvent to dryness, the residual solid was recrystallized from *i*-PrOH to give 2.8 g (76%); mp 213–214°C; (found C, 54.80; H, 7.13; N, 18.55; Cl, 9.50; calc $C_{17}H_{28}ClN_5O_2$ requires C, 55.20; H, 7.63; N, 18.94; Cl 9.58%). IR, cm^{-1}: 1690, 1685, 1660, 1555. ^{1}H-NMR: 8.03 s

(1H, = C^8-H), 4.44 t (2H, J = 6.9 Hz, $-N^7-CH_2-$); 3.54 s (3H, $N-CH_3$); 3.36 s (3H, $N-CH_3$); 3.33, 3.22 m (3H, $-CH_2-N-$ and $-N-CH-$); 2.80 s (3H, $N-CH_3$); 2.35 t (2H, J = 7.6 Hz, $-CH_2-$), 1.99, 1.18 m (10H, cyclohexyl).

Biological evaluation

The experiments were conducted on 40 guinea pigs weighing 500–600 g, 290 male white rats of the Wistar line, weighing 180–250 g, and 210 male white mice weighing 18–22 g. Acute toxicity (LD_{50}) of compounds **3a** and **3b** was assessed by dissolving in saline (0.9% NaCl) with 1–2 drops of Tween 80, and administering to mice and rats *via* oral (*op*), intraperitoneal (ip) and intravenous (iv) routes. The LD_{50} was evaluated at 4 or 5 different doses, each on 6 animals and calculated by the method of Litchfield–Wilcoxon, using a personal Pravetz–8M computer.

Under urethane anesthesia (1.2 g/kg ip) the guinea pigs were made to respire artificially with a small-animal ventilator. The bronchoconstriction in the animals was recorded using a bronchospasm transducer (Ugo Basile, Comerio Varese, Italy) by an overflow technique described by Konzett and Rossler [14], as modified by Kaminka [15]. Compounds **3a** and **3b** were injected intravenously into *v jugularis* in the form of 2% solution at dose 1/5, 1/10 and 1/20 of iv LD_{50} for rats.

The broncholytic effect of the compounds was likewise evaluated during *in vitro* experiments on isolated guinea-pig trachea according to Castillo and de Beer [16]. For this purpose, the organ isolated was immersed in an organic bath (cup) of 30 ml volume with Krebs-Henseleit solution, at temperature 37°C and carbogen aeration. Tracheal contractions induced by acetylcholine (1×10^{-6} M) were registered by means of an isotonic transducer and a Unirecord writing device, obtained from Ugo Basile Co (Italy). Cumulative concentration–effect curves of tested compounds were plotted, and the mean effective concentrations EC_{50} and pD_2 were calculated using the regression analysis method [17]. The results underwent statistical processing by the Student–Fischer t-test at $p \leq 0.05$.

The compounds' effect on phosphodiesterase activity was also assessed and compared to that of aminophylline determined by the modified method of Butcher and Sutherland [18] in rat lung and heart, based on the measurement of inorganic phosphorus by the method of Buell [19].

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