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ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JANUARY 1994

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

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(Received 16 July 1993; accepted 13 December 1993)

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 ¹H-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

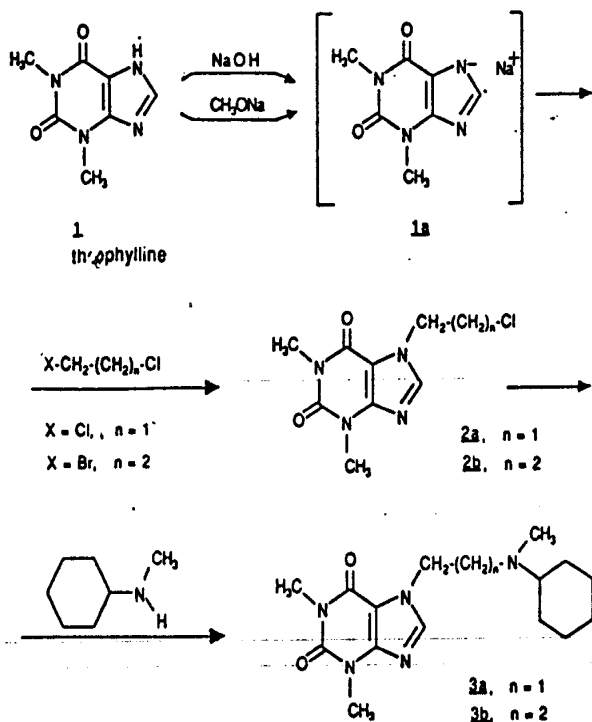
Theophylline and its derivatives possess bronchodilatory activity. The most widely used drug of this group is aminophylline, which is a soluble complex formed between theophylline and ethylenediamine [1]. However, this drug exhibits some side effects including severe allergic reactions and cardiotoxicity [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 (LD₅₀) of 3a and 3b, as compared to aminophylline, showed that compounds 3a and 3b had acute toxicity (LD₅₀) close to the standard substance aminophylline (table I).

As shown by *in vivo* experiments (table II) or 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 LD₅₀ *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 (*p*D₂ = 4.54) is statistically more significant than that exerted by

Table I. Acute toxicity (LD₅₀)* of compounds 3a, 3b and aminophylline.

Compound	Animal	Administration route	LD ₅₀ (mg/kg) (range of values)
3a	mouse	ip	206.9 (162.7 – 262.9)
	mouse	op	598.1 (495.2 – 722.4)
	rat	iv	116.5 (87.9 – 154.3)
	rat	ip	206.9 (162.7 – 262.9)
	rat	op	2880.0 (2293.0 – 3618.0)
3b	mouse	ip	116.5 (87.9 – 154.3)
	mouse	op	299.1 (229.0 – 390.6)
	rat	iv	102.5 (72.6 – 144.9)
	rat	ip	116.5 (87.9 – 154.3)
	rat	op	1639.0 (1331.1 – 2018.3)
aminophylline	mouse	iv	125.0 (111.2 – 141.1)
	mouse	ip	180.1 (158.6 – 205.3)
	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) <i>iv</i>	Decrease of acetylcholine (10 mg/kg)-induced bronchospasm in the guinea pig (%)			
		2 min	10 min	20 min	30 min
3a	6	53.8 ± 5.4	34.6 ± 4.3	—	—
	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
3b	5	22.7 ± 3.2	—	—	—
	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 $M \pm SD$ ($n = 6$); * $p \leq 0.05$ 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 3×10^{-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 (%)
3a ^a	1×10^{-6}	0
	3×10^{-6}	6.1 ± 2.6
	1×10^{-5}	$27.7 \pm 7.8^*$
	3×10^{-5}	$61.8 \pm 7.7^*$
	1×10^{-4}	$100.0 \pm 0^*$
	3×10^{-4}	—
3b	1×10^{-6}	0
	3×10^{-6}	0
	1×10^{-5}	0
	3×10^{-5}	0
	1×10^{-4}	6.9 ± 7.3
	3×10^{-4}	31.3 ± 6.2
aminophylline ^b	1×10^{-6}	0
	3×10^{-6}	4.8 ± 1.8
	1×10^{-5}	12.2 ± 3.4
	3×10^{-5}	37.2 ± 7.0
	1×10^{-4}	63.3 ± 6.4
	3×10^{-4}	100 ± 0

The data were presented in $M \pm SD$ ($n = 6$); * $p \leq 0.05$ statistically significant difference in comparison with aminophylline. ^a3a: $EC_{50} = 2.9 \times 10^{-5}$ mol/l, $r = 0.962$, $pD_2 = 4.54$; ^baminophylline: $EC_{50} = 7.1 \times 10^{-5}$ 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 2×10^{-3} M and 2×10^{-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 2×10^{-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 1×10^{-5} , 3×10^{-5} and 1×10^{-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 2×10^{-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}	$88.3 \pm 4.3^*$	$94.2 \pm 5.8^*$
	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 $M \pm SD$ 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 Miyamoto *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 $^{\circ}C$ and were corrected. 1H -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-Chloroethyl)-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 $78-80^{\circ}C$ 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 were dried over anhydrous sodium sulfate and after filtration the solvent was removed. The product 2a crystallized from ethanol in 90% yield, mp: $120-122^{\circ}C$ [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 $151-152^{\circ}C$ [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 $100^{\circ}C$ for 285 min. After cooling to $50^{\circ}C$, 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 $181.5-183^{\circ}C$; (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. 1H -NMR: 8.04 s (1H, C⁸-H); 4.33 t (2H, $J = 4.1$ Hz, $-N-CH_2-$); 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, $-N-CH_2-$, 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 $50^{\circ}C$. 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 $213-214^{\circ}C$; (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. 1H -NMR: 8.03 s

(1H, = C⁸-H), 4.44 t (2H, $J = 6.9$ Hz, -N⁷-CH₂-); 3.54 s (3H, N-CH₃); 3.36 s (3H, N-CH₃); 3.33, 3.22 m (3H, -CH₂-N- and -N-CH₂-); 2.80 s (3H, N-CH₃); 2.35 t (2H, $J = 7.6$ Hz, -CH₂-), 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 (LD₅₀) 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 LD₅₀ 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* LD₅₀ 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 EC₅₀ and pD₂ 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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