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Synthesis and antibronchospastic activity of theophylline thioacetal derivatives

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theophylline derivatives / antibronchospastic mucolytic

Introduction

Theophylline and its derivatives [1, 2, 3] are well-known for their bronchodilator activity and consequent efficacy in the treatment of bronchial asthma.

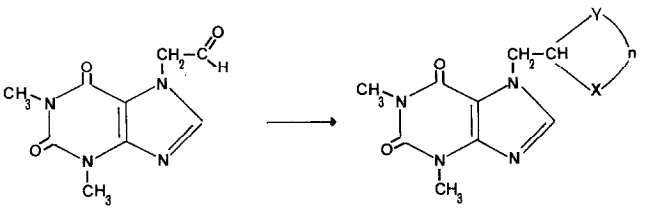
Related N_7-substituted theophylline derivatives such as proxyphylline [4], bamiphylline [5], diphylline [6], and doxophylline [7, 8] are also in therapeutic use. Among these, doxophylline (Ansimar®), was shown to be effective in reducing bronchial muscle spasm, its activity being greater than that of theophylline, showing very poor side effects on cardiovascular and central nervous systems [9] due to a lack of affinity between this drug and A1 and A2 adenosine receptors [10–12].

As a part of our research on antibronchospastic compounds, we prepared some doxophylline thioanalogues with the aim of synthesizing a compound showing 2 complementary pharmacodynamic activities, *i.e.* mucolytic and antibronchospastic. Indeed the dithiocycloalkyl side chain could bestow the mucolytic activity on the theophylline molecule, as has previously been demonstrated for other sulfur containing compounds [13–15].

Chemistry

The general synthetic methods employed to prepare compounds **II–IV** involved cyclothioacetalyzation (Scheme 1) of 7-theophyllineacetaldehyde **(I)**.

While dithioacetals **II, III** were obtained under BF_3-etherate catalysis, compound **IV** was prepared in the presence of $ZnCl_2$. The 7-theophylline-1,3-dithiolane-1-oxide diastereomers **(V, VI)** were synthesized in good yields form **II** by H_2O_2 oxidation in acetic acid and the separation of the 2 diastereomers was achieved by using flash-chromatography. Other cyclic sulfoxide diastereomers were separated in this way [17].



I

	n	X	Y
II	$-(CH_2)_2-$	S	S
III	$-(CH_2)_3-$	S	S
IV	$-(CH_2)_2-$	O	S
V–VI	$-(CH_2)_2-$	S	S=O

Scheme 1.

The stereochemical assignment was obtained according to the following evidence: i) the *trans* isomer exhibited the signals of methylene group ($\geq N-CH_2-$) at 4.65 and 4.45 δ upfield with respect of signals of *cis* isomer. This is in good agreement with the observations of Carey [18]. ii) the *cis* isomer, upon heating in aprotic solvent (dimethylsulfoxide), was smoothly transformed into the corresponding dihydro-1,4-dithiin derivative whereas the *trans* isomer was not. This is in accord with Chen's results [19].

The structures of all mentioned new products were confirmed by analytical IR, UV, NMR, and MS spectral data.

Results and Discussion

The data obtained from the pharmacological studies indicate compound **II** as the most interesting of the tested compounds.

In fact it showed a antihistaminic action equivalent to that of aminophylline in the guinea-pig trachea test (ED_{50} 164 μg/ml, Table I) and greater antibronchospastic

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activity (ED_{50} 5.1 mg/kg, Table II) than compounds **III, IV, V, VI** and reference compounds.

Furthermore, compound **II** significantly increased the production of tracheobronchial mucus in the rabbit after oral treatment, its potency being markedly higher than that of other known mucokinetic drugs, used as reference (bromhexyne, *N*-acetylcysteine, Table III). On the contrary doxophylline, the 1,3 dioxolane theophylline derivative, was completely devoid of this effect.

Table I. *In vitro* antibronchospastic activity of compounds **II**–**VI** and reference compounds on guinea-pig isolated trachea.

Compound	IC_{50} (μg/ml)	Confidential limits
II	164.7	92–294
III	496.4	350–502
IV	300.2	250–380
V	352.6	246–504
VI	384.2	280–484
Doxophylline	215.4	105–312
Aminophylline	206.1	117–363

Due to the lack of free thiol group in the molecule, this compound probably behaved as an indirectly acting (blocked thiol group) mucolytic, its target being the syalil-transferases enzymes [24].

All the tested compounds did not present significant oral toxicity with LD >900 mg/kg as shown in Table IV.

Compound **II** was therefore selected for further pharmacological and clinical studies.

Experimental protocols

Chemistry

Chemicals

7-Theophyllineacetaldehyde was prepared from 7-(2,3-dihydroxy-propyl)-theophylline according to Toffoli *et al.* [16]. 1,2-ethandithiol, 1,3-Propandithiol, 1,2-mercaptoethanol, *p*-toluensulfonic acid, boron trifluoride etherate, 35% hydrogen peroxide and dioxane were from E. Merck (Darmstadt, FRG). Analytical grade chemicals and solvents were used as received without purification.

The reported melting points are uncorrected.

Spectral studies

^1H NMR spectra were recorded in $CHCl_3$ on a FT JEOL GX270 spectrometer using tetramethylsilane as internal standard.

IR and UV spectra were recorded on 781 Perkin–Elmer and U-3200 double-beam Hitachi, respectively. Mass spectra were from a Kratos MS-80 (Electron impact).

Syntheses

7-(1,3-Dithiolan-2-ylmethyl)theophylline ***II.*** 1,2-Ethandithiol 5 ml

Table II. Inhibitory effect of theophylline thioacetal derivatives on acetylcholine-induced bronchospasm in anaesthetized guinea-pigs.

Compound	Dose (mg/kg i.v.)	% Inhibition (mean ± SD)	ED_{50} (mg/kg i.v.)
II	2.5	26.5 ± 4.8	5.35
	5.0	49.4 ± 6.5	
	10.0	68.0 ± 5.4	
III	2.5	5.0 ± 2.8	>15.00
	5.0	17.3 ± 6.0	
	10.0	29.0 ± 6.3	
	15.0	41.1 ± 6.5	
IV	5.0	14.5 ± 3.2	15.20
	10.0	34.7 ± 7.7	
	15.0	47.3 ± 7.1	
V	2.5	3.1 ± 2.1	>15.00
	5.0	11.8 ± 4.9	
	10.0	26.0 ± 4.5	
	15.0	36.1 ± 5.5	
VI	2.5	7.8 ± 3.7	>15.00
	5.0	14.3 ± 5.9	
	10.0	24.5 ± 9.5	
	15.0	37.2 ± 8.4	
Doxophylline	2.5	6.5 ± 3.5	10.85
	5.0	26.7 ± 5.7	
	10.0	47.4 ± 6.4	
Aminophylline	2.5	10.1 ± 3.9	10.90
	5.0	25.9 ± 9.1	
	10.0	48.8 ± 7.4	

(5.6 g, 0.059 mol) and 10 ml boron trifluoride etherate were added to a warm solution of 7-theophyllineacetaldehyde (10 g, 0.045 mol) in 350 ml dry dioxane. After 5 h of heating at 60°C under stirring, the dioxane was evaporated under reduced pressure and 400 ml $CHCl_3$ were added. The solution was thoroughly washed with 5% aqueous NaOH (2 × 200 ml) and shaken with saturated brine (2 × 200 ml).

The organic phase was then dried over anhydrous sodium sulphate and the solvent evaporated. The crude product was purified by flash-chromatography on silica gel eluting with CH_2Cl_2 and then with CH_2Cl_2/EtOH (99/1) to give 6.6 g **II** (50% yield). mp: 125–127°C from methanol (white needles).

^{1}H NMR (270 MHz) ($CDCl_3$, δ): 3.25 (s, 4H, CH_2CH_2), 3.40 (s, 3H, N_3-CH_3), 3.59 (s, 3H, N_1-CH_3), 4.20 (d, 2H, CH_2CH, J = 7 Hz), 4.93 (t, 1H, CH_2CH, J = 7 Hz), 7.66 (s, 1H, N=CH). IR ($CHCl_3$): 1705, 1660, 1550 cm^{-1}. UV (CH_3OH): λ_{max} = 275, 204 nm. MS (EI): *m/z*: 298 (M^+, 12.4), 180 (35.5), 119 (56.1), 118 (69.5), 105 (100).

7-(1,3-Dithian-2-ylmethyl)theophylline ***III.*** The synthesis was performed as described for **II**. mp: 154–156°C from methanol (white needles).

^{1}H NMR (270 MHz) ($CDCl_3$, δ): 2.00 (m, 2H, $CH_2CH_2CH_2$), 2.74–2.92 (m, 4H, $2CH_2-S$), 3.40 (s, 3H, N_3-CH_3), 3.58 (s, 3H, N_1-CH_3), 4.44 (t, 1H, CH_2CH, J = 7.4 Hz), 4.65 (t, 2H, CH_2CH, J = 7.4 Hz), 7.66 (s, 1H, N=CH). IR ($CHCl_3$): 1705, 1660, 1605, 1550 cm^{-1}. UV (CH_3OH): λ_{max} = 275, 205 nm. MS (EI): *m/z:* 312 (M^+, 20.6), 180 (23.2), 134 (15.2), 133 (32.5), 132 (100), 119 (78.9).

7-(1,3-Oxathiolan-2-ylmethyl)theophylline ***IV.*** The synthesis was performed as described for compound **II** using $ZnCl_2$ instead of boron trifluoride to give **IV** (6.1 g, 48%) which after crystallization from methanol melted at 113–115°C.

^{1}H NMR (270 MHz) ($CDCl_3$, δ): 2.86–3.04 (m, 2H, SCH_2), 3.38 (s, 3H, N_3-CH_3), 3.57 (s, 3H, N_1-CH_3), 3.80–3.89 (m, 1H, OC*H*), 4.24–4.31 (m, 1H, OC*H*), 4.52 (dd, 1H, CH_a-CH_c, JH_aH_b = 14 Hz, JH_aH_c = 6.6 Hz), 4.66 (dd, 1H, CH_b-CH_c, JH_bH_c = 3.3 Hz, JH_aH_b = 14 Hz), 5.43 (dd, 1H, CH_2-CH_c, JH_aH_c = 3.3 Hz, JH_bH_c = 6.6 Hz), 7.64 (s, 1H, N=CH). IR ($CHCl_3$): 1705, 1660, 1605, 1550 cm^{-1}. UV (CH_3OH): λ_{max} = 275, 205 nm. MS (EI): *m/z*:282 (M^+, 13.2), 194 (33.1), 193 (6.5), 180 (31.7), 103 (83.6), 89 (100).

7-(1,3-Dithiolan-2-ylmethyl)theophylline-1S-oxide ***V, VI.*** 2 ml 35% H_2O_2 were added to a solution of 7-(1,3-dithiolan-2-ylmethyl)theophylline **II** (7 g, 0.031 mol) in 75 ml acetic acid.

The mixture was stirred for 5 h at room temperature then 500 ml diethyl ether was added to induce precipitation.

After overnight storage at −30°C, the precipitate was filtered and the 2 diastereomers were separated by flash-chromatography on a silica-gel column: elution with CH_2Cl_2/CH_3OH 97.5/2.5 gave the *cis* isomer, followed by the *trans* isomer on eluting with CH_2Cl_2/CH_3OH (96.5/3.5) mixture (64% yield, 55:45 *trans:cis*).

The 2 diastereomers melted with decomposition at 204–206°C (CH_3OH) for the *trans* isomer and at 200–202°C (CH_3OH) for the *cis* isomer.

trans-isomer: ^{1}H NMR (270 MHz) ($CDCl_3$, δ): 2.98 (m, 1H, 1,3 dithiolane ring), 3.41 (s, 3H, N_3-CH_3), 3.56 (m, 2H, 1,3 dithiolane ring), 3.61 (s, 3H, N_1-CH_3), 3.80 (m, 1H, 1,3 dithiolane ring), 4.45 (dd, 1H, CH_a-CH_c), 4.65 (m, 2H, CH_b-CH_c), 7.73 (s, 1H, CH=N). IR ($CHCl_3$): 1700, 1655, 1550 cm^{-1}. UV (CH_3OH): λ_{max} = 275, 207 nm. MS (EI): *m/z:* 314 (M^+, 76.9), 298 (12.2), 297 (79.1), 286 (56.6), 238 (36.1), 237 (11.9), 206 (26.8), 205 (100), 193 (14.3), 181 (17.7), 180 (81.0).

cis-isomer: ^{1}H NMR (270 MHz) ($CDCl_3$, δ): 2.84 (m, 1H, 1,3-dithiolane ring), 3.41 (s, 3H, N_3-CH_3), 3.45 (m, 1H, overlapped, 1,3-dithiolane ring), 3.61 (s, 3H, N_1-CH_3), 3.83 (m, 1H, 1,3-dithiolane ring), 4.64 (dd, 1H, CH_a-CH_c, JH_aH_b = 14 Hz, JH_aH_c = 8.6 Hz), 4.82 (dd, 1H, CH_b-CH_c, JH_aH_b = 14 Hz, JH_bH_c = 6 Hz), 5.0 (dd, 1H, $CH_aH_b-CH_c$, JH_aH_c = 8.6 Hz), 7.75 (s, 1H, CH=N). IR ($CHCl_3$): 1700, 1655, 1550 cm^{-1}. UV (CH_3OH) λ_{max} = 275, 207 nm. MS (EI): *m/z:* 314 (M^+, 87.4), 298 (13.2), 297 (85.3), 286 (59.4), 238 (37.8), 237 (12.4), 206 (26.5), 205 (100), 181 (17.3), 180 (82.3)

Thermal rearrangement of ***V***

0.5 g of **V** were dissolved in 20 ml of anhydrous dimethylsulfoxide and heated at 100°C for 64 h. The reaction was then diluted with 100 ml H_2O and extracted with $CHCl_3$ (3 × 60 ml). The residue was purified by preparative TLC with $CHCl_3$ as eluant.

^{1}H NMR (270 MHz) ($CDCl_3$, δ): 3.33 (2H, m, $-CH_2-S-$), 3.42 (3H, s, N_3-CH_3), 3.46 (2H, m, $-CH_2-S-$), 3.60 (3H, s, N_1-CH_3), 6.56 (1H, s, $-CH=C$), 7.66 (1H, s, $-CH=N-$). MS (EI): *m/z:* 296 (M^+, 100), 263 (38.2), 181 (71.9), 180 (68.1), 116 (56.3).

Table IV. Acute toxicity in mice following oral administration of theophylline derivatives.

Compound	LD_{50} (mg/kg)
II	1200
III	900
IV	980
V	900
VI	900

Table III. Mucoproduction in rabbits before and after oral treatment with **II**, doxophylline, bromhexyne and *N*-acetylcysteine.

Substance	*n*	Dose (mg/kg)	Mucoproduction (mg/h) (mean ± SD)		% Variation with respect to its own base	Statistical significance with respect to its own base
			−4–0 h (before treatment)	0–4 h (after treatment)		
Control	10	–	29.50 ± 8.32	31.25 ± 5.93	+ 5.93	N.S.
	10	10	30.60 ± 9.43	34.87 ± 10.25	+13.95	N.S.
II	10	30	27.20 ± 5.22	34.53 ± 4.16	+26.95	$P<0.05$
	10	100	29.70 ± 4.95	46.71 ± 8.82	+57.27	$P<0.01$
Doxophylline	10	100	29.80 ± 5.80	32.10 ± 8.26	+ 7.71	N.S.
Bromhexyne	10	400	28.90 ± 7.82	39.01 ± 4.34	+34.98	$P<0.05$
N-acetylcysteine	10	400	27.70 ± 5.50	32.19 ± 3.89	+16.21	$P<0.05$

Pharmacology

In vitro *activity on guinea-pig trachea smooth muscle*
Male guinea-pig trachea was allowed to function *in vitro* in 50 ml Krebs' solution, at 37°C under suitable oxygenation.

The lower part of the trachea was closed, while the upper part was connected to a small polyethylene tube leading to a transducer to measure volume variations. Both the trachea and the polyethylene tube were filled with Krebs' solution and the responses to histamine (5 μg/ml) were determined before and after the addition of theophylline derivatives at increasing concentrations.

In vivo *studies on smooth muscles*
The method of Konzett and Rossler [20] was utilized with minor modifications [21]. Male guinea-pigs weighing 300–450 g, under urethane anaesthesia (1.0 g/kg, i.p.), were used.

The jugular vein of each animal was cannulated for intravenous administration of drugs, while the trachea was connected to pump for artificial respiration (Palmer pump for small animals: stroke frequency 70 insufflations per minute).

The pneumogram was recorded on polygraph (Ugo Basile, Milano, Italy).

When the animals were stabilized, a bronchospasm was stimulated with acetylcholine (0.2 mg/kg i.v.).

After 2 similar responses to spasm inducing injections, theophylline derivatives were administered (0.02–10 mg/kg, i.v.) followed, 2 to 5 min later, by the bronchoconstrictor drug.

The effects of the tested compounds were evaluated with reference to the percentage reduction of the induced bronchoconstriction.

Acute toxicity
Male Swiss mice weighing 18–22 g were used. Test compounds suspended in 0.5% carboxymethylcellulose, were administered orally.

The LD_{50} values were calculated according Litchfield and Wilcoxon [22], 10 days after test compounds were administered.

The maximal toxicity was observed after 24 h, when the animals showed decreased muscle tone and laboured respiration signs.

Mucolytic activity
For this test a modified method described by Perry and Boyd [23], was used. Groups of 10 New Zealand male, while rabbits, weighing 2.8–3.5 kg, were used.

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