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Original article

Structure–activity relationships in a series of xanthine derivatives with antibronchoconstrictory and bronchodilatory activities

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Summary — Thirty-one 1,3,7,8-substituted xanthine derivatives have been synthesized and evaluated for bronchodilator and antibronchoconstrictory activities in *in vitro* tracheal relaxation and *in vivo* bronchospasm inhibition models. Activity tests have been complemented with phosphodiesterase inhibition and toxicological data. Structure–activity relationships are discussed. Compound **21** (1,3-diisobutyl-8-methylxanthine) has been selected for further pharmacological development because of its good activity profile and favourable therapeutic index, which is 14- and 38-fold greater than that of theophylline and IBMX, respectively.

Résumé — Relations structure–activité dans une série de nouveaux dérivés de la xanthine montrant une activité antibroncho-constrictrice et bronchodilatatrice. *Trente et un dérivés de la xanthine 1,3,7,8-substituée ont été synthétisés et évalués pour leur activité broncho-dilatatrice et antibroncho-constrictrice dans des modèles de relâchement trachéen* in vitro *et d'inhibition du bronco-spasme* in vivo. *Les tests d'activité ont été complétés par l'inhibition de la phosphodiestérase et des données toxicologiques. Les rapports structure–activité sont discutés. Le composé* **21** *(1,3-diisobutyl-8-méthylxanthine) a été choisi pour un développement pharmacologique ultérieur, car il jouit d'un bon profil d'activité et d'un indice thérapeutique favorable, 14 et 38 fois plus grand que celui de la théophylline et de l'IBMX, respectivement.*

xanthine derivatives / bronchodilatory / antibronchoconstrictory activity / phosphodiesterase inhibition

Introduction

Theophylline has been widely used in the therapy of bronchospastic diseases including acute asthma attacks, chronic bronchitis and emphysema [1, 2]. Nevertheless, it has important drawbacks and a low therapeutic margin, associated with a pharmacokinetic profile highly influenced by individual factors which lead to the need of monitoring blood levels and which makes its use difficult. Thus, the active investigation of new xanthine derivatives with improved pharmacodynamic and/or pharmacokinetic properties is still to be developed.

With the aim of obtaining new compounds with a wider safety margin, we were encouraged to pursue the preparation of a series of xanthine derivatives with different substituents at the 1, 3, 7 and 8 positions of the purine nucleus. The activity of the compounds was monitored by the following *in vitro* and *in vivo* tests: spontaneous tracheal relaxation and inhibition of histamine-induced bronchospasm in guinea pigs. LD_{50} in mice was calculated as an index of acute toxicity. Since some effects of methylxanthines may be due to inhibition of cyclic nucleotide phosphodiesterase, the activity of this enzyme was measured in the presence of these compounds.

Chemistry

The synthesis of xanthines **1–31** was performed according to the general method summarized in scheme 1.

Unsymmetrical dialkylureas were prepared by conventional reaction of the corresponding alkyl isocyanate with an alkylamine. Symmetrical dialkylureas were obtained in a more straightforward fashion by reaction of diethylcarbonate with 2 equivalents of the alkylamine, following the procedure of Wawzonek [3]. The appropriate 1,3-dialkyl-6-aminouracil was prepared according to the method of Papesch and Schroeder [4]. Nitrosation, reduction and cyclization were performed by the procedure described by Kramer *et al* [5]. The synthesis of xanthine derivatives carrying a substituent in position 7 was performed by

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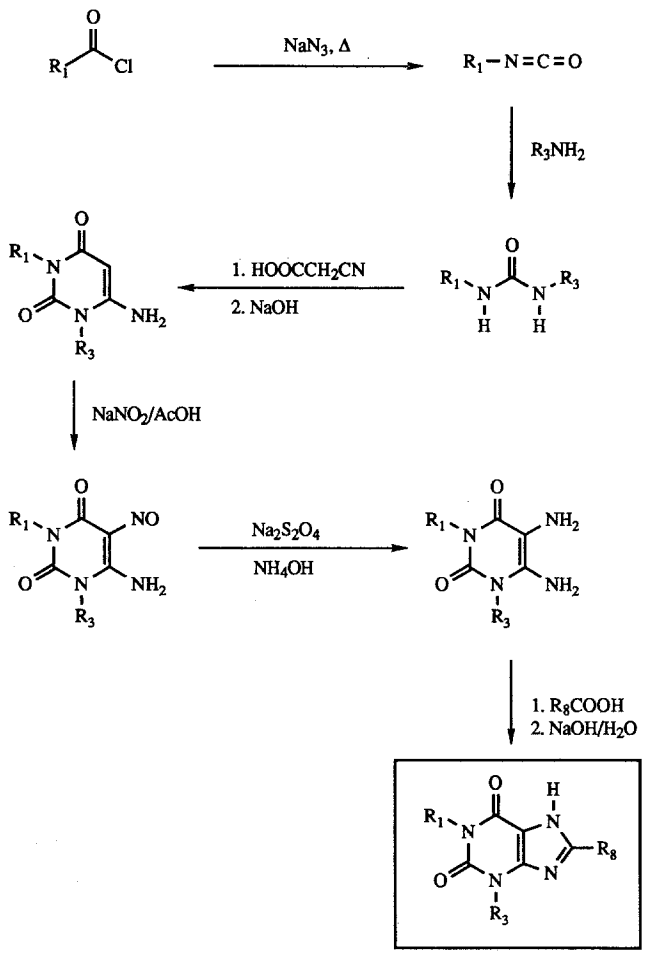


Scheme 1.

treatment of the parent xanthine with potassium carbonate in DMF and reaction with an alkylating agent, according to Garst *et al* [6].

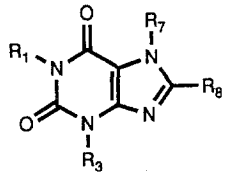
Results and Discussion

The results of both *in vitro* and *in vivo* pharmacological tests are shown in table I. An exhaustive analysis of the data has been made to establish possible structure–activity relationships. In a general manner, the replacement of the 3-methyl group of theophylline by alyphatic radicals, branched or linear, led to a considerable increase in both activity and toxicity. Preferred substituents were propyl, butyl, isobutyl, cyclopropylmethyl and cyclohexylmethyl (**2**, **4**, IBMX, **5**, **6**). On the other hand, substitution by aromatic radicals such as phenyl, benzyl or phenylethyl (**8–10**) provided enhanced *in vitro* but not *in vivo* activity, which suggests that aromaticity in this position produces an impairment of bioavailability. These results are in accordance with those found in the literature, emphasizing the importance of the 3-substitution in bronchodilatory potency and toxicity [7, 8]. Moreover, the introduction of a methyl group at the 8-position of 1,3-disubstituted xanthines yielded more potent and toxic compounds (**11** *vs* IBMX, **18** *vs* **6**, **16** *vs***10**) [9]. Further substitution at the same position by an ethyl group (**12**, **17**, **19**) or by a bulkier alkyl chain (**13–15**) produced a decrease in activity. Another important observation was the loss of activity and toxicity when substitutions were made at the 7-position of IBMX (**23–31**). Otherwise, replacement of a methyl group by an isobutyl group at the 1 position of 3-isobutylxanthines produced a considerable decrease in phosphodiesterase inhibitory activity (IBMX, **11**, **12** *vs* **20**, **21** and **22**, respectively) but minor changes in the *in vitro* and *in vivo* activity tests were noted.

Relationships between pharmacological and toxicological data have been established as shown in table II. Taking the data as a whole, there was a fair correlation between tracheal relaxation and toxicity, and even a good correlation was found between the latter and the inhibition of bronchospasm *in vivo*. Interestingly, there was a lack of correlation between the 2 classical *in vitro* and *in vivo* tests for bronchodilatory activity, *ie* tracheal relaxation and bronchospasmic inhibition, which could be attributed to a different pharmacokinetic behaviour of the tested compounds. Furthermore, there was no correlation between phosphodiesterase inhibition and activity *in vitro* and *in vivo*, supporting the hypothesis that increasing intracellular cAMP levels are not the main molecular mechanism to explain the bronchodilatory activity of xanthines [10–12], and suggesting the important role of other regulatory systems such as adenosine antagonism [13] and calcium channel blockade [14]. Nevertheless, the role of phosphodiesterase inhibition cannot be excluded, as this enzyme exists in multiple forms, depending on the organ and of affinity towards cAMP and other cyclic nucleotides, mainly cGMP. In fact, it has been recently demonstrated that there is a high degree of correlation between bronchodilatory activity of some xanthine derivatives and inhibition of chromatographically-purified phosphodiesterase fractions from dog tracheal muscle preparations [15].

In conclusion, we have developed a series of new xanthine derivatives with enhanced bronchodilatory and antibronchoconstrictory activities in comparison with theophylline and, in some cases, with a better therapeutic index. It is worth mentioning compound **21**, which possesses a potency of about 250– and 14–fold that of theophylline in the tracheal relaxation test and in the bronchospasm inhibition assay, respectively. In spite of its high pharmacological activity, compound **21** showed a relatively mild acute toxicity in mice (240 mg/kg, ip), which leads to a therapeutic

Table I. Structure-activity relationships in a series of substituted xanthine derivatives.



compd.	R_1	R_3	R_8	R_7	mp, °C	recrystn. solvent	formula	anal.	PDE inhibn. K_i μM	Trachea relaxn. EC_{50} μM	Bronchospasm inhibn. ID_{50} mg/kg,ip	LD50 mg/kg,ip
1	H	Me	H	H	>330	H_2O	$C_6H_6N_4O_2$	C,H;N[a]	130	55	100	894
2	Me	*n*-Pr	H	H	220-222	H_2O	$C_9H_{12}N_4O_2$	C,H;N[b]	7.5	0.2	2.5	70
3	Me	*i*-Pr	H	H	215-216	MeOH	$C_9H_{12}N_4O_2$	C,H,N	29	5.2	10	-
4	Me	*n*-Bu	H	H	207-214	H_2O	$C_{10}H_{14}N_4O_2$	C,H,N	55	1.1	2.5	237
5	Me	CH_2-*c*-Pr	H	H	215-219	MeOH	$C_{10}H_{12}N_4O_2$	C,H,N	1.5	1.0	1.0	75
6	Me	CH_2-*c*-Hex	H	H	228-230	MeOH	$C_{13}H_{18}N_4O_2$	C,H,N	5.9	0.31	2.9	320
7	Me	CH_2-*t*-Bu	H	H	234-235	MeOH	$C_{11}H_{16}N_4O_2$	C,H,N	7.2	2.8	2.5	204
8	Me	Ph	H	H	>325	EtOH	$C_{12}H_{10}N_4O_2$	C,H,N	11	0.2	96	470
9	Me	Bn	H	H	263-264	MeOH	$C_{13}H_{12}N_4O_2$	C,H,N	6.1	0.8	35	392
10	Me	$(CH_2)_2$Ph	H	H	206-218	EtOH/H_2O	$C_{14}H_{14}N_4O_2$	C,H,N	6.1	3.8	100	445
11	Me	*i*-Bu	Me	H	220-227	H_2O	$C_{11}H_{16}N_4O_2$	C,H,N	1.7	0.09	1.2	25
12	Me	*i*-Bu	Et	H	215-219	H_2O	$C_{12}H_{18}N_4O_2$	C,H,N	0.5	0.4	2.0	46
13	Me	*i*-Bu	*n*-Pr	H	194-195	Me_2CO	$C_{13}H_{20}N_4O_2$	H,N;C[c]	1.1	1.6	15	214
14	Me	*i*-Bu	*n*-Bu	H	160-163	EtOH/H_2O	$C_{14}H_{22}N_4O_2$	H,N;C[d]	0.6	0.9	15	340
15	Me	*i*-Bu	*i*-Bu	H	172-174	MeOH	$C_{14}H_{22}N_4O_2$	C,H,N	5.7	1.9	20	339
16	Me	$(CH_2)_2$Ph	Me	H	257-260	EtOH/H_2O	$C_{15}H_{16}N_4O_2$	C,H,N	2.5	5.5	20	83
17	Me	$(CH_2)_2$Ph	Et	H	210-225	Me_2CO	$C_{16}H_{18}N_4O_2$	C,H,N	1.4	3.8	>50	396
18	Me	CH_2-*c*-C_6H_{11}	Me	H	253-255	MeOH	$C_{14}H_{20}N_4O_2$	C,H,N	1.5	0.2	2.2	208
19	Me	CH_2-*c*-C_6H_{11}	Et	H	190-192	MeOH	$C_{15}H_{22}N_4O_2$	C,H,N	0.7	1.7	5	426
20	*i*-Bu	*i*-Bu	H	H	192-195	EtOH/H_2O	$C_{13}H_{20}N_4O_2$	C,H,N	35	9.1	1.5	796
21	*i*-Bu	*i*-Bu	Me	H	247-249	MeOH	$C_{14}H_{22}N_4O_2$	C,H,N	74	0.12	0.3	240
22	*i*-Bu	*i*-Bu	Et	H	223-225	MeOH	$C_{15}H_{24}N_4O_2$	H,N;C[e]	33	2.4	5	322
23	Me	*i*-Bu	H	CH_2Ph	112-115	MeOH	$C_{17}H_{20}N_4O_2$	H,N;C[f]	2.4	11	110	>1000
24	Me	*i*-Bu	H	CH_2-*p*-Cl-C_6H_4	100-101	EtOH/H_2O	$C_{17}H_{19}ClN_4O_2$	C,H,N,Cl	1.7	32	150	503
25	Me	*i*-Bu	H	CH_2CHO	125-126	MeCN	$C_{12}H_{16}N_4O_3$	C,H,N	140	83	10	-
26	Me	*i*-Bu	H	$CH(OMe)_2$	112-114	MeOH	$C_{14}H_{22}N_4O_4$	C,H,N	81	95	50	956
27	Me	*i*-Bu	H	$(CH_2)_2CH(OEt)_2$	79-80	-	$C_{16}H_{26}N_4O_4$	H,N;C[g]	82	130	>100	>1000
28	Me	*i*-Bu	H	$CH_2CH(OCH_2)_2$	126-127	MeOH	$C_{14}H_{20}N_4O_4$	C,H,N	72	2.8	50	1000
29	Me	*i*-Bu	H	$CH_2CHOHCH_2OH$	155-158	EtOH	$C_{13}H_{20}N_4O_4$	H,N;C[h]	290	20	23	784
30	Me	*i*-Bu	H	CH_2COOH	190-200	H_2O	$C_{12}H_{16}N_4O_4.H_2O$	C,H,N	170	34	55	>1000
31	Me	*i*-Bu	H	$(CH_2)_2COOH$	162-175	H_2O	$C_{13}H_{18}N_4O_4$	C,H,N	36	1700	70	>1000
Theophylline	Me	Me	H	H					140	31	4.9	241
IBMX	Me	*i*-Bu	H	H					3.7	1.1	2.5	44

[a]N 33.72, found 30.22; [b]N 26.91, found 27.35; [c]C 59.07, found 59.69; [d]C 60.45, found 59.93; [e]C 61.62, found 62.11; [f]C 65.37, found 64.35; [g]C 54.54, found 53.92; [h]C 52.69, found 51.57.

Table II. Correlation analysis among the results of the different pharmacological tests. Calculated value was r (Pearson correlation coefficient). $n = 31$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	PDE inhibition	*Tracheal relaxation*	*Bronchospasm inhibition*	LD_{50}
PDE Inhibition	—	0.032	0.096	0.463**
Tracheal relaxation	0.032	—	0.206	0.345*
Bronchospasm inhibition	0.096	0.206	—	0.608***
LD_{50}	0.463***	0.345*	0.608***	—

index (LD_{50}/ID_{50}) of about 660 in bronchospasm. This is 14– and 38–fold greater than that of the reference compounds theophylline and IBMX, respectively. This compound is now under further pharmacological development.

Experimental protocols

Chemistry

Melting points were determined with a Mettler FP80 central processor melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 983 spectrophotometer; 80 MHz ^1H and 20.1 MHz ^{13}C NMR spectra were recorded on a Bruker AC 80 spectrometer and are reported in ppm on the δ scale from the indicated reference. Combustion analyses were performed using a Carlo Erba 1106 analyzer. Liquid chromatography was performed using a forced flow (flash chromatography) of the indicated solvent system on SDS silica gel Chromagel 60 ACC (230–400 mesh). Analytical thin layer chromatography (TLC) was performed using Magery–Nagel 0.25 mm silica gel SIL G-25 plates. Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values.

General synthesis of xanthines. Preparation of xanthine ***21***

N,N'-*diisobutylurea*
A slight modification of the method of Wawzonek [3] was followed. To a cooled (0°C) solution of isobutylamine (146.3 g, 2 mol) in water (90 ml), diethylcarbonate (214.2 g, 1 mol) was slowly added in portions. The 2 phase solution was mechanically stirred at room temperature for 18 h. Water (6 l) was added, and volatiles removed under reduced pressure. When the remaining volume was *ca* 1 l, the solution was allowed to cool to room temperature. The pinkish-white resulting precipitate was filtered, washed with cold water, and dried under vacuum for 48 h (P_2O_5, 50°C) to afford 164.2 g (95%) of product. mp = 121–129°C; IR (KBr) ν 3355, 2953, 2867, 1627, 1574, 1465, 1385, 1271, 1055, 669 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 5.6 (br s, 2H, NH), 2.96 (d, J = 6.2 Hz, 4H, 2 NCH_2), 1.71 (multiplet, J = 6.8 Hz, 2H, 2 $CHMe_2$), 0.88 (d, J = 6.8 Hz, 12H, 2 $CHMe_2$); ^{13}C NMR (20 MHz, $CDCl_3$) δ ($CDCl_3$) 159.50, 47.87, 29.10, 20.12. Anal $C_{10}H_{20}N_2O$ (C, H, N).

1,3-Diisobutyl-4-aminouracil
To a solution of *N,N'*-diisobutylurea (160 g, *ca* 0.93 mol) in acetic anhydride (335 ml) cyanacetic acid was added (93.2 g, 1.09 mol). The red solution was stirred at 70°C for 2 h, and then the excess of acetic anhydride was distilled off under reduced pressure at a temperature not higher than 60°C. The oily residue was treated with 10% aqueous sodium hydroxide (370 ml). Upon solution of the urea, a spontaneous increase in temperature (65–70°C) was produced, and the formed uracil started to precipitate. Stirring was maintained without heating for an additional hour, and then the reaction mixture was cooled to 0°C in order to complete precipitation. The precipitate was filtered, washed thoroughly with cold water, and dried at 45°C under vacuum. The crude product (215 g, 97%) was a pale greyish–yellow solid that could be directly used without further purification (mp 97–98°C). An analytical sample was obtained by a 2–fold recrystallization from ethyl acetate, as a white crystalline solid. mp = 134–136°C; IR (KBr) ν 3349, 3208, 2959, 2868, 1694, 1615, 1490, 1405, 1278, 787, 554 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 5.52 (br s, 2H, NH_2), 5.01 (br s, 1H, =CH), 3.72 (d, J = 7.5 Hz, 4H, NCH_2), 2.11 (multiplet, J = 6.8 Hz, 2H, 2 $CHMe_2$), 0.95 (d, J = 6.8 Hz, 6H, $CHMe_2$), 0.87 (d, J = 6.8 Hz, 6H, $CHMe_2$); ^{13}C NMR (20 MHz, $CDCl_3$) δ ($CDCl_3$) 163.38, 154.37, 152.26, 75.51, 49.39, 47.95, 27.66, 27.15, 20.06, 19.05. Anal $C_{12}H_{21}N_3O_2$ (C, H, N).

1,3-Diisobutyl-4-amino-5-nitrosouracil
To a cooled (5°C) stirred mixture containing 1,3-diisobutyl-4-aminouracil (214 g, *ca* 0.89 mol) in water (2 l) and acetic acid (108 ml) was added $NaNO_2$ (68 g, 0.98 mol) in small portions. A deep purple color, characteristic of the nitroso derivative appeared immediately, and stirring was continued at room temperature for 2 h. The mixture was then cooled in an ice bath, and stirred for 1 additional hour. The solid was filtered off, washed with cold water, and dried at 45°C under vacuum to afford 226 g (94%) of a crystalline, deep purple solid. The crude product was pure enough to be used in the next step without further purification. mp 222–224°C; IR (KBr) ν 3198, 2958, 2871, 1720, 1673, 1511, 1409, 1235, 1114, 753 cm^{-1}; ^1H NMR (80 MHz, DMSO–d_6) δ (TMS) 3.76 (d, J = 7.2 Hz, 4H,

NCH_2), 2.00 (m, 2H, 2 $CHMe_2$), 0.89 (d, J = 6.6 Hz, 6H, $CHMe_2$), 0.87 (d, J = 6.6 Hz, 6H, $CHMe_2$); ^{13}C NMR (20 MHz, $CDCl_3$) δ ($CDCl_3$) 160.59, 149.65, 145.73, 138.75, 47.60, 47.07, 26.68, 25.81, 19.88, 19.27. Anal $C_{12}H_{20}N_4O_3$ (C, H, N).

1,3-Diisobutyl-4,5-diaminouracil

To a stirred mixture containing finely divided 1,3-diisobutyl-4-amino-5-nitrosouracil (225 g, *ca* 0.84 mol) in 25% aqueous ammonia (1.26 l), sodium dithionite (511 g, 2.9 mol) was added in small portions, the temperature raised to 35–36°C, and the purple colour disappeared gradually. When no more increase temperature was observed, the reaction mixture was stirred at 50°C for 1 additional hour. At this moment, the purple color had almost totally disappeared, and the mixture was then allowed to stand overnight at room temperature, in order to complete the reaction (total disappearance of the color). The 1,3-diisobutyl-4,5-diaminouracil precipitated from the ammonia solution, and the reaction mixture was then cooled in an ice-bath to achieve the total precipitation. Finally, the solid was filtered off, thoroughly washed with cold water, and dried at room temperature under vacuum over NaOH pellets. The crude product was used without further purification. The yield was 188 g (88%) of a brownish–grey solid, showing an ill-defined melting point at ≈ 110–130°C. IR (KBr) ν 3350, 3219, 2959, 2871, 1688, 1646, 1603, 1489, 1273, 1100, 762 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 5.21 (br s, 2H, NH_2), 3.77 (d, J = 7 Hz, 2H, NCH_2), 3.75 (d, J = 7 Hz, 2H, NCH_2), 2.5 (br s, 2H, NH_2), 2.14 (m, J = 7 Hz, 2H, 2 $CHMe_2$), 0.97 (d, J = 7 Hz, 6H, $CHMe_2$), 0.89 (d, J = 7 Hz, 6H, $CHMe_2$); ^{13}C NMR (20 MHz, $CDCl_3$) δ ($CDCl_3$) 161.7, 150.83, 148.7, 95.42, 49.9, 48.3, 27.7, 27.1, 20.0, 19.8. Anal $C_{12}H_{22}N_4O_2$ (C, H, N).

1,3-Diisobutyl-8-methylxanthine ***21***

1,3-Diisobutyl-4,5-diaminouracil (188 g, *ca* 0.74 mol) was mixed with vigorous stirring with acetic acid (613 ml, 10.7 mol). The mixture was refluxed for 2 h, resulting in a clear reddish solution. The excess of acetic acid was distilled off under reduced pressure. To the residue was added absolute ethanol (60 ml), and evaporated *in vacuo*. This was repeated 3 times, in order to completely eliminate the excess of acetic acid. The residual brown paste was suspended with vigorous stirring in a 2.2 M aqueous solution of sodium hydroxide (1 l), and heated to reflux for 45 min, as a result of which most of the solid was dissolved. The reaction mixture was allowed to cool to 40°C with stirring, and was then filtered by suction, in order to eliminate the non-soluble impurities. To the resulting clear solution was added a little decoloring carbon, and filtered again. Then, the xanthine was precipitated by the addition of hydrochloric acid until the pH was 2–3. The suspension was cooled to 0°C to complete precipitation. Finally, the solid was filtered off, thoroughly washed with cold water, and dried under vacuum at 50°C over sodium hydroxide, to afford 165 g (80%) of product as a buff colored solid, found to be pure by TLC analysis (mp 234–236°C). Recrystallization from methanol afforded the title product as a white solid. mp 240.9–241.0°C; IR (KBr) ν 3144, 3088, 3045, 2959, 2867, 1707, 1646, 1558, 1503, 1284, 1091, 1014, 816, 764 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 3.97 (d, J = 7.5 Hz, 2H, NCH_2), 3.93 (d, J = 7.5 Hz, 2H, NCH_2), 2.58 (s, 3H, $=CCH_3$), 2.30 (m, 2H, 2 $CHMe_2$), 0.96 (d, J = 6.6 Hz, 12H, 2 $CHMe_2$); ^{13}C NMR (20 MHz, $CDCl_3$ + DMSO-d_6) δ (TMS) 154.1, 151.3, 150.2, 148.3, 106.3, 49.8, 47.3, 26.8, 26.7, 19.9, 19.7, 14.33. Anal $C_{14}H_{22}N_4O_2$ (C, H, N).

Alkylation of the 7-position. General procedure

The method of Garst *et al* was followed [6]: to a mixture of the xanthine (1 eq) and anhydrous potassium carbonate (1.2 eq) in dry dimethylformamide the corresponding alkyl halide was added (1.1 eq). The mixture was stirred between room temperature and 80°C until TLC analysis indicated total disappearance of the starting xanthine. The solvent was removed *in vacuo* and the solid residue was partitioned between water and dichloromethane. The product was isolated from the organic phase and recrystallized from the appropriate solvent.

Pharmacology

Phosphodiesterase inhibition

Enzyme activity was determined using purified phosphodiesterase from beef heart (specific activity 0.25 U/mg, Boehringer Mannheim), according to the spectrophotometric method of Menahan *et al* [16].

Spontaneous relaxation of guinea pig tracheal strips

Male albino guinea pigs weighing 400–500 g were killed by a blow on the head. Trachea was rapidly excised and prepared according to Emmerson and MacKay [17]. The organ was suspended in an organ bath containing Krebs solution and loaded with 0.5 g. Spontaneous relaxation with cumulative doses of tested compound was recorded by using an isometric transducer Ugo Basile 7003. Isoprenaline 10^{-6} M provided 100% relaxation. The EC_{50} value — the concentration of the drug required to relax trachea by 50% — was calculated.

Protection against histamine-induced bronchoconstriction in the anesthetized guinea pig

Male albino guinea pigs weighing 450–500 g were anesthetized with urethane (1.5 g/kg, *ip*). After suppression of spontaneous breathing with gallamine (5 mg/kg, *iv*) the animal was connected to a Harvard ventilator (56 strokes/min). Bronchoconstriction was recorded with a bronchospasm transducer Ugo Basile 7020 according to Konzett and Rössler [18]. When repetitive histamine response was achieved, the tested compound was administered intraperitoneally 30 min before a new histamine challenge, and the ID_{50} value was calculated.

Acute toxicity

The LD_{50} value was calculated after intraperitoneal administration to Swiss albino female mice according to Litchfield and Wilcoxon [19].

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