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Original article

Synthesis and bronchospasmolytic effects of benzocyclobutenic and benzocyclenic compounds

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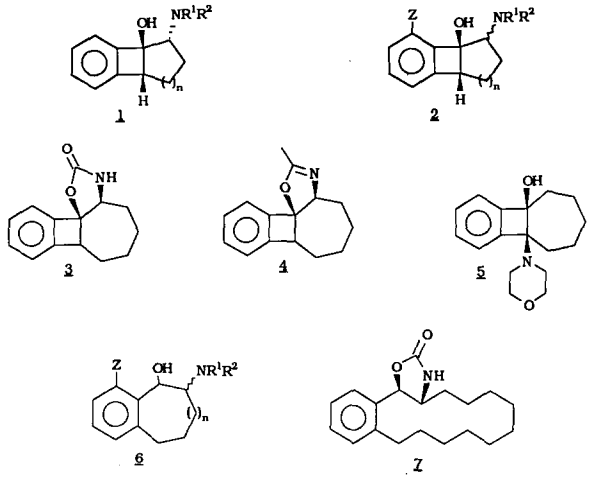
Summary — Two series of new benzocyclobutenic and benzocyclenic compounds were synthesized and tested for bronchospasmolytic effects. Theophylline was used as reference. Compound **1b** was found to be 12.6 to 15.8-fold more potent *in vitro* than theophylline and devoid of antagonistic effects towards adenosine.

Résumé — Synthèse et propriétés bronchospasmolytiques de dérivés benzocyclobuténiques et benzocycléniques. Deux séries de nouveaux dérivés benzocyclobuténiques et benzocycléniques ont été synthétisés et testés dans le but d'étudier leur activité bronchospasmolytique. La théophylline a été utilisée comme substance de référence. Le produit **1b** apparaît 12,6 à 15,8 fois plus actif que la théophylline et est dépourvu d'activité antagoniste vis-à-vis de l'adénosine.

benzocyclobutene / benzocyclene / bronchodilators

Introduction

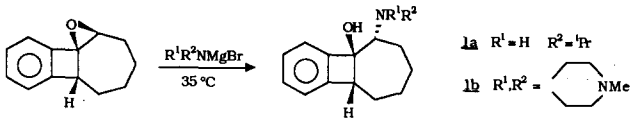
In the present study we extended our former study concerning the bronchospasmolytic effects of compounds **1** [1, 2] to a series of similar substrates **2** in which the relative positions of the hydroxyl and amino groups are either *cis* (symbol *2 cis*) or *trans* (symbol *2 trans*). We also explored the activities of **3** and **4** (2 derivatives of these alcohols) as well as of **5**, the first element of a new family of aminobenzocyclobutenols.



In the same way, were studied compounds **6** and **7** which belong to the benzocyclenic series.

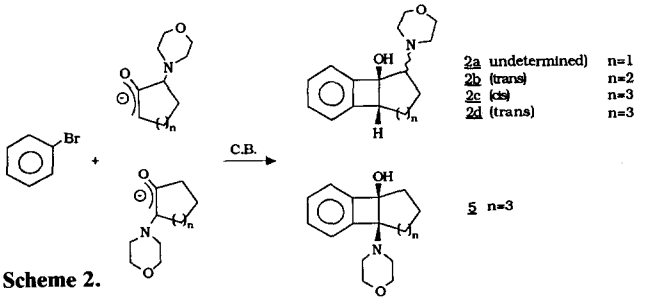
Chemistry

The synthesis of **1a** and **1b** (scheme 1) was performed by condensation of the corresponding magnesium amides on the appropriate oxiranne [1, 2].



Scheme 1.

The amino-alcohols **2a–d** and **5** were obtained by arynic condensation of alpha amino-ketones on bromobenzene in the presence of complex bases (CB) as previously published [3] (scheme 2). Note that the spectroscopic data were never published and are given in the *Experimental protocols*.



Scheme 2.

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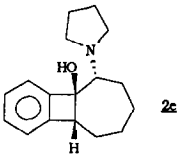
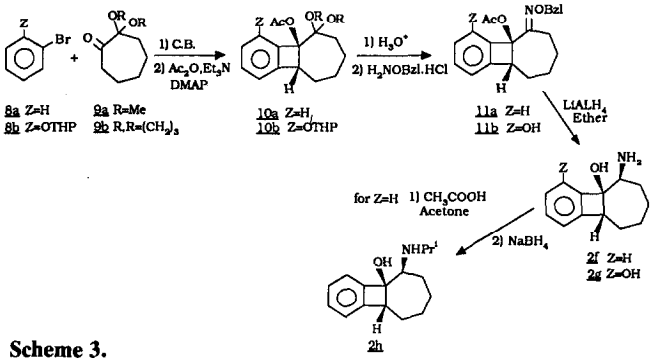
The new product **2e** *trans* (Z = H; R^1, R^2 = $(CH_2)_4$; *n* = 3) was obtained in the same way:

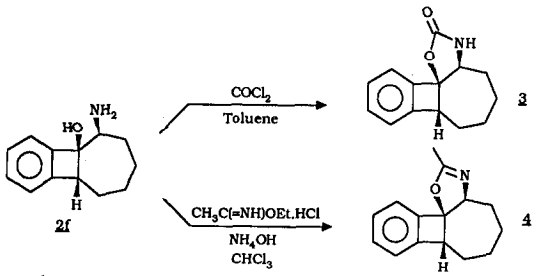


Compounds **2f** (Z = H), **2g** (Z = OH), and **2h** were obtained according to scheme 3.



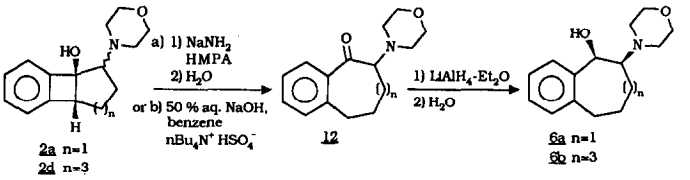
Scheme 3.

Products **3** and **4** were obtained from the corresponding amino-alcohol **2f** according to scheme 4.



Scheme 4.

Products **6a** and **6b** were obtained from the corresponding benzocyclobutenols according to scheme 5.



Scheme 5.

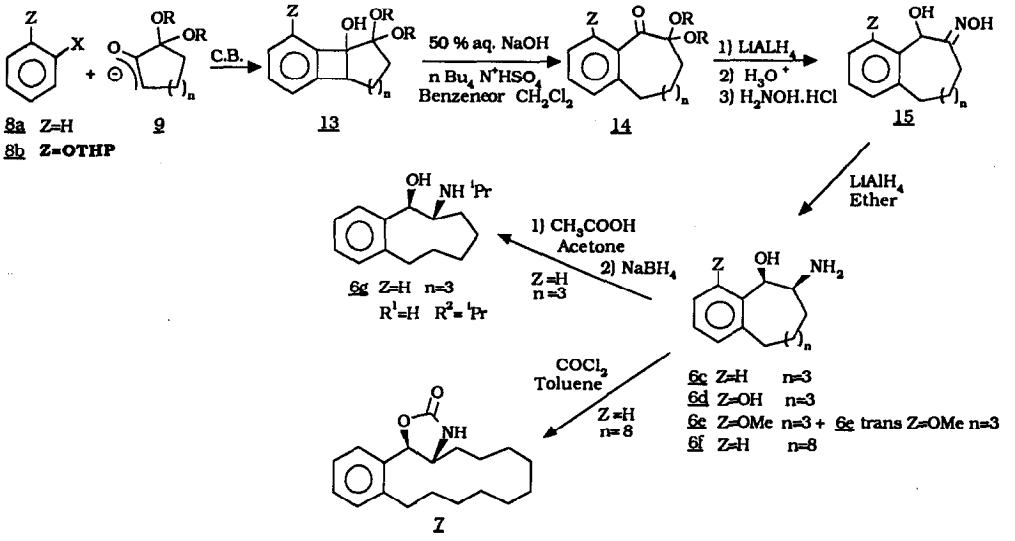
Finally, products **6c–g** and the derivative **7** were prepared according to scheme 6 [4].

The structures of all the new products were established by their spectroscopic data given in the *Experimental protocols*.

Pharmacological results and Discussion

The effects of theophylline and of the compounds, tested *in vitro* on the isolated guinea pig trachea model and quantified by measuring pD_2 (-log EC_{50}), are shown in table I. It appears that **1b** is the most active of the new compounds, being respectively 15.8 and 12.6-fold more potent than theophylline *versus* histamine and acetylcholine. Then come, in decreasing order of activity against histamine:

2d *trans n* = 3 > **2h** *cis* > **6b** *cis n* = 3 ≥ **2c** *cis n* = 3 ≥ **2b** *trans n* = 2 and in descending order of activity against acetylcholine: **2c** *cis n* = 3 > **6b** *cis n* = 3 ≥ **2d** *trans n* = 3 ≥ **2h** *cis* Z = H > **6f** *cis n* = 8 = **6g** ≥ 5 > **2g** *cis* Z = OH; **2d** *trans n* = 3 > **2h** *cis* > **6b** *cis n* = 3 ≥ **2c** *cis n* = 3 ≥ **2b** *trans n* = 2; **2b** *trans n* = 2 ≥ **1a**, **6c**



Scheme 6.

Table I. –log EC_{50} (pD_2) of theophylline and **1a–12b** compounds *versus* histamine and acetylcholine on the guinea pig isolated trachea. Values are mean ± SEM (experiments = 3).

		pD_2 vs *Hist*	$\frac{x}{Theo}$	pD_2 vs *ACh*	$\frac{x}{Theo}$
Theophylline		4.08 ± 0.10	–	3.51 ± 0.06	–
1a		3.75 ± 0.12	0.47	3.55 ± 0.14	1.10
1b		5.28 ± 0.09	15.80	4.61 ± 0.11	12.60
2a	$n = 1$	< 3.5	–	< 3.5	–
2b *trans*	$n = 2$	4.25 ± 0.09	1.48	3.87 ± 0.09	2.29
2c *cis*	$n = 3$	4.27 ± 0.11	1.54	4.50 ± 0.13	9.77
2d *trans*	$n = 3$	4.80 ± 0.08	5.24	4.35 ± 0.11	6.92
2e *trans*	$n = 3$	< 3.5	–	< 3.5	–
2g *cis*	$n = 3$	3.68 ± 0.12	0.40	3.95 ± 0.09	2.75
2h *cis*		4.34 ± 0.07	1.81	4.33 ± 0.16	6.61
3		< 3.5	–	< 3.5	–
4		< 3.5	–	< 3.5	–
5		3.67 ± 0.14	0.39	4.16 ± 0.12	4.47
6a *cis*	$n = 1$	< 3.5	–	< 3.5	–
6b *cis*	$n = 3$	4.33 ± 0.13	1.78	4.37 ± 0.09	7.24
6c *cis*	$n = 3$	< 3.5	–	3.74 ± 0.08	1.69
6e *trans*	$n = 3$	3.55 ± 0.11	0.29	3.60 ± 0.08	1.23
6e *cis*	$n = 3$	< 3.5	–	3.57 ± 0.11	1.14
6f *cis*	$n = 8$	4.10 ± 0.14	1.04	4.15 ± 0.15	4.36
6g		4.20 ± 0.14	1.31	4.15 ± 0.14	4.36
7		< 3.5	–	< 3.5	–

cis $n = 3$, **6e** *trans* $n = 3$, **6e** *cis* $n = 3$ which are much more or slightly more active than theophylline.

The **1a**, **5**, **6e** *trans* $n = 3$, **2g** *cis* Z = OH compounds are less active than theophylline *versus* histamine, and the **2a**, **7**, **4**, **3**, **2e** *trans* $n = 3$ and **6a** *cis* $n = 1$ compounds are inactive ($pD_2 < 3.5$) against both histamine and acetylcholine.

Complementary studies were performed with **1b**. This compound had relaxant effects on the isolated guinea pig trachea contracted with KCl 10 mM. The pD_2 values for **1b** and theophylline were 4.71 and 3.60 respectively, which gives a **1b**/theophylline ratio of 12.9, a figure similar to that obtained *versus* acetylcholine and histamine.

In vivo, the doses of **1b** and theophylline that reduced by 50% the bronchoconstriction induced in guinea pig by histamine and acetylcholine were 7.8 ± 2.4 and 14.4 ± 3.1 mg/kg (experiments = 6) respectively with **1b** and 12.2 ± 2.1 and 28.4 ± 2.7 mg/kg (experiments = 6) respectively with theophylline. Thus, **1b** proved to be 1.56 and 1.97-fold more active than theophylline in this experimental model. The 100% lethal dose of **1b** on anaesthetized guinea pigs was 30.0 ± 1.6 mg/kg (experiments = 12).

Finally, **1b** did not shift to the right the concentration–action curve of adenosine on the isolated guinea pig trachea model, thus contrasting with theophylline which has been shown to exert a competitive antagonism towards adenosine (pA = 5.07, regression slope = 0.84) (fig 1).

1b therefore appears to behave as a spasmolytic compound of the papaverine type (*ie* acting directly on smooth muscle). It is approximately 10–15-fold more potent than theophylline and is devoid of antagonistic effect towards adenosine. This last property makes **1b** similar to emprofylline which is also

devoid of this antagonistic effect. However, **1b** seems to be more active than emprofylline on the isolated guinea pig trachea model, since the latter is only 3.8 and 4.55-fold more active than theophylline *versus* histamine and acetylcholine respectively [5], whereas the corresponding figures for **1b** are 15.8 and 12.6 respectively.

As regards structure–activity relationships, our results show that in the benzocyclobutenic series the activity of the compounds is dependent: a) on the size of the saturated ring: a cycloheptenic ring is more active than a cyclohexenic ring (**2d** > **2b**); b) on the kind of amine used: with the same carbon skeleton and the same stereochemistry, *N*-methylpiperazine induces a greater effect than morpholine which itself induces a much greater effect than pyrrolidine (**1b** > **2d** ≥ **2e**); c) on the position of the amine group: the activity falls when the amine group is located at the junction of the ring. Thus, compound **5** has a lower activity than **2d** *trans* $n = 3$; d) on the type of heterocycle formed by including the hydroxyl and amine group: the transformation of the α-amino-alcohol chain into oxazoline and oxazolidinone results in inhibition; e) on the presence of a substituent on the aromatic ring: hydroxy or methoxy substituent on the aromatic ring only leads to compounds with very low activity.

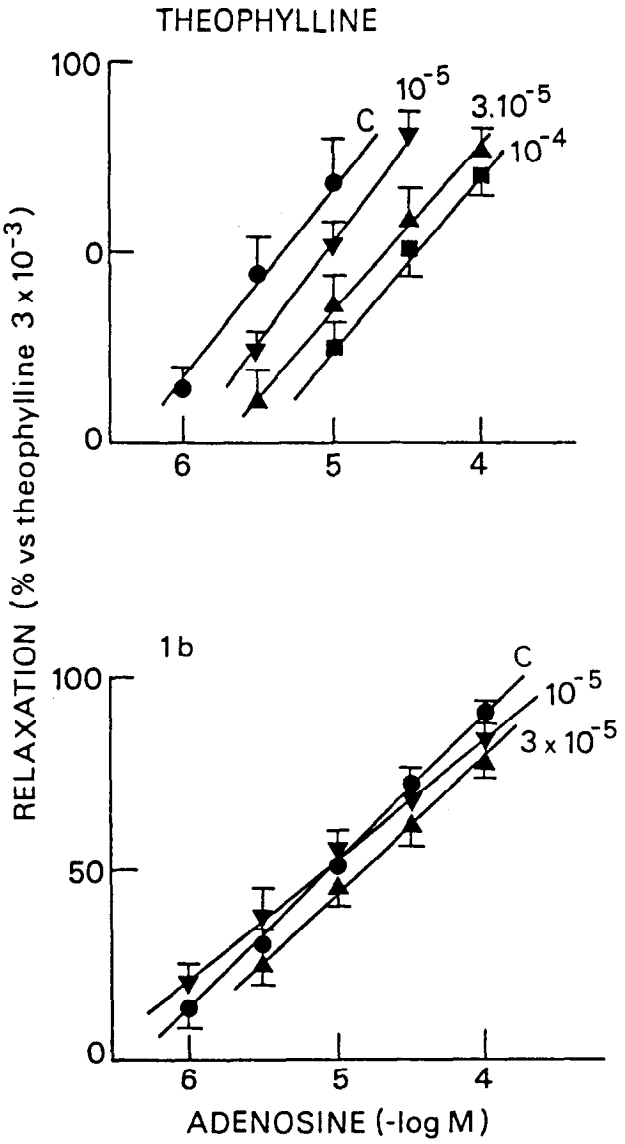


Fig 1. Concentration relaxant–response curve to adenosine on the guinea pig isolated trachea in the absence (C) or in the presence of theophylline (10^{-5}–10^{-4} M) or **1b** (10^{-5} and 3×10^{-5} M). Experiments were performed in the presence of dipyridamole 10^{-5} M. Values are mean ± SEM (experiments = 5).

The activity observed in the benzocyclenic series primarily depends on 2 factors: a) amine substitution: a tertiary amine (*eg* morpholine) induces a greater effect than a secondary amine ($NHPr^i$) which itself induces a greater effect than a primary amine (NH_2); b) the size of the ring bearing the OH and NR^1R^2 groups. The observed activity increases with the size of the non-aromatic ring; thus, the phenylethanolamine derivative with a 14-carbon ring has greater activity than the 9-carbon ring. The position of the OH and NR^1R^2 has no effect on the detected activity.

We conclude that in the benzocyclobutenic series the compound **1b** with a saturated 7 carbon atoms ring and a *N*-methylpiperazine group has, beside its *in vivo* toxicity, highly interesting properties: it is more active than theophylline and emprofylline and is devoid of antagonistic effects towards adenosine. On the other hand in the benzocyclenic series, the activity increases with the size of the non-aromatic ring (9 → 14 atoms).

Experimental protocols

Chemistry

General methods

Melting points were determined on a Köfler melting point apparatus. ^{13}C NMR-spectra were recorded on Brüker WP 80 and Brüker AM 400 spectrometers, ^1H NMR spectra on a Perkin–Elmer R 12 B instrument at 60 MHz, on a Brüker AW 80 instrument at 80 MHz and on a Brüker AM 400 instrument at 400 MHz with Me_4Si as internal standard. Ultraviolet spectra were obtained with methanol solutions on a Beckman Model DK 2A instrument. Infrared spectra with NaCl film or KBr pellets were recorded on a Perkin–Elmer 580 instrument.

Elemental analyses were performed by CNRS laboratory (Vernaison) and by M François (Strasbourg). Thin-layer chromatography was performed by using Kieselgel G (Merck) with a hexane–EtOAc mixture as eluent. The silica gels used for

liquid phase chromatography and flash chromatography were respectively Kieselgel 0.063 (0.2 mm) and Kieselgel 0.04 (0.063 mm). Preparative high-performance liquid chromatography (HPLC) was carried out on a Waters PREP 500 chromatograph with a silica gel column (47 x 300 mm 55-105 μm). Analytical HPLC was performed in a Waters model 6000 A instrument with a stainless steel column Merck Hibar RT 250-4 (Lichrosorb Si 60-5 μM).

Materials

Degussa sodamide was washed with appropriate solvents and finely ground with a mortar under solvent. Tetrahydrofuran (THF) freshly distilled from a benzophenone-sodium couple, stored under sodium was used, hexamethylphosphoramide (HMPA) was distilled before use, dichloromethane was distilled from P_2O_5. The starting benzocyclobutenols **1** were prepared as previously described [6]. **1a** and **1b**; see [1, 2]. **2a–d** and **5**; see [3] for preparation and table II for characteristic physical data.

*Synthesis of compound **2e***

The enolate preparation with LDA–diisopropylamine (16 mM) in THF (20 ml) under dry nitrogen at 0°C was treated with *n*-BuLi (1.6 M; 10 ml). After 15 min, the α aminoketone **2** (15 mM) in THF (10 ml) was added. To a CB prepared as above ($NaNH_2$/Bu^tONa = 60 mM/30 mM for the ratio PhBr/**2** = 1/1) was added the lithio enolate then the bromobenzene (15 mM in the ratio PhBr/**2** = 1/1) at 25°C and for 0.5 h. Upon completion, the mass was poured on ice, extracted with Et_2O, washed twice with H_2O, and dried over $MgSO_4$. After evaporation of the solvents under reduced pressure, **2e** was purified by preparative HPLC.

*Synthesis of compound **2h***

10a was obtained by arynic condensation of the enolate **9a** (R = Me) on bromobenzene [6], however; and instead of the usual work-up described in [5], the mixture was degassed by blowing through a stream of nitrogen for half an hour, then the mixture was decanted and the supernate taken off with a transfer line and added dropwise to a solution of acetic anhydride (15.3 g; 150 mM) Et_3N (6.7 g; 26 mM) and DMAP (1 g; 85 mM) in THF (20 ml). At the end of the reaction (monitoring by TLC), the solution was poured on ice, extracted with Et_2O and dried over $MgSO_4$. Solvents were evaporated under vacuum. After purification on HPLC, the compound **10a** was obtained (9 g; 31 mM; 62% yield with respect to the bromobenzene) IR (NaCl): ν 1750 cm^{-1} ($OCOCH_3$); ^1H NMR (CCl_4): δ 1.04–2.84 (11 H, m with s at 1.91, $OCOCH_3$ and 4 CH_2), 3.15 and 3.33 (6 H, 2 s, 2 OCH_3), 3.56–4.04 (1 H, m, benzylic H), 6.78–7.47 (4 H, m, aromatic H); UV (MeOH) λ nm (log ε): 274 (3.28), 267.5 (3.31), 262 (3.18).

11a was obtained by removing the ketal function with 3 drops of HCl 50% in acetone. After usual work-up and purification by preparative HPLC the carbonyl compound thus obtained in quantitative yield was transformed into the corresponding benzyloxime following the procedure described in the literature [7] (85% yield) IR (NaCl): ν 1755 ($OCOCH_3$), 1610 cm^{-1} (C=N-OCH_2Ph); ^1H NMR (CCl_4): δ 1.07–2.83 (11 H, m with 2 s at 1.8 and 1.97, $OCOCH_3$ (*syn* and *anti*) and 4 CH_2), 5.55 and 5.02 (2 H, 2 s, OCH_2 *syn* and *anti*), 6.96–7.62 (9 H, m with s at 7.21, aromatic H); UV (MeOH) λ nm (log ε): 273 (3.39), 266 (3.43), 259 (3.36). MS: $C_{15}H_{16}O_3$ *m/e* = 244. Compound **11a** (1.74 g; 5 mM) was reduced by $LiAlH_4$ (1.52 g; 40 mM) in ether to yield (0.9 g; 4.45 mM; 89%) of **2f** *cis* Z = H. mp 114°C (EtOAc-PE); IR (KBr): ν 3360 (OH), 3700–2500 cm^{-1} (NH_2); ^1H NMR (CCl_4): δ 0.62–2.09 (11 H, m, 4 CH_2, OH and NH_2 exchanged with D_2O), 2.86–3.19 (2 H, m, C*H*NH_2, benzylic H), 6.91–7.42 (4 H, m, aromatic H); UV (MeOH) λ nm (log ε): 260 (2.97), 266 (3.10), 272 (3.09). ^{13}C NMR ($CDCl_3$) δ (aromatic) 148.85, 146.14, 129.09, 127.38, 122.64, 120.64 (aliphatic) 83.69 (C-OH), 56.71 (Ph-*C*H), 56.30 (*C*NH_2), 33.46, 30.95, 29.41, 27.02 (4 CH_2); Anal calcd for $C_{13}H_{17}NO$: C, 76.82; H, 8.40; N, 6.80. Found: C, 77.17; H, 8.77; N, 6.91.

The alkylation was performed following the procedure described in the literature [8] and afforded 70% yield after purification by flash chromatography of the desired compound **2h**. Physical data are given in table II.

*Synthesis of **2g***

To the enolate of **9b** (1 eq) prepared as previously published [6] and then cooled at 15–20°C, was added 2-(*p*-chlorophenoxy)tetrahydropyran (1.1 eq) in THF. Stirring was continued for 3 h at this temperature. Protection reaction of the benzocyclobutenic hydroxyl and the subsequent work-up were carried out as above. Separation of the mixture was performed by preparative HPLC (eluent: 25% EtOAc/petroleum ether) to afford the acetate **10b** in 55% yield.

Removal of the protecting group was performed in acetone at 0°C with 3 drops of concentrated HCl (89% yield) to give the keto acetate intermediate.

After recovery of the carbonyl group, oximation and reduction was performed as above to give first **11b** and finally the amino-alcohol **2g** with an overall yield for the 3 steps of 31%.

*Synthesis of **3** and **4***

The amino-alcohol **2f** (570 mg; 2.8 mM) was dissolved in 0.25 normal aqueous potassium hydroxide (13.7 ml); potassium carbonate (1.8 g) was added to the solution which was covered with toluene (4 ml). Then a 20% solution of phosgene in toluene (5 ml) was added dropwise at 0°C. The mixture was stirred for a further 2 h. The mixture was allowed to warm to room temperature, poured into water and extracted with ether (3 x 30 ml). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was rapidly chromatographed on silica gel (eluent: 40% EtOAc/petroleum ether) to give **3** (588 mg; 2.56 mM; 92% yield). See table II for physical data.

To a magnetically stirred solution of the amino-alcohol **2f** (700 mg; 3.44 mM) and $CH_3C(=NH)OEt.HCl$ (852 mg; 6.89 mM) in $CHCl_3$ (40 ml) were added 10 drops of 33% aqueous NH_4OH solution. After 48 h, the mixture was poured into water. After extraction with CH_2Cl_2 (3 x 100 ml), drying over $MgSO_4$ and removal of the solvents under vacuum, the residue was chromatographed on silica gel (eluent: 40% EtOAc/petroleum ether) to afford **4** (690 mg; 3.04 mM; 89%). See table II for physical data.

*Synthesis of **6a** cis and **6b** cis*

The amino-alcohol **2a** (500 mg; 2.04 mM) was dissolved in benzene (10 ml) with 5% equivalent of $nBu_4N^+HSO_4^-$ then 50% aqueous NaOH (10 ml) were added and the mixture was vigorously stirred at room temperature for 15 min. The 2 phases were decanted and the aqueous layer was extracted twice with ether. After drying over $MgSO_4$ and evaporation of the solvent *in vacuo*, the amino ketone **12a** was rapidly isolated by chromatography (eluent: 25% EtOAc/petroleum ether) on a short column (400 mg; 1.63 mM; 80% yield).

The above amino ketone dissolved in ether (20 ml) was reduced with $LiAlH_4$ (130 mg; 3.5 mM) at ambient temperature. At the completion of the reaction (30 min; monitored by

Table II. Physical data for benzocyclobutenic amino-alcohols and derivatives. [a]On the hydrochloride compound with dioxane as internal reference, 67.4 ppm downfield from TMS.

Compounds	mp, °C (solv.)	Molecular formula	Analyses Calcd % Found % C	H	N	IR (solv.) ν, cm^{-1}	UV (MeOH) λ, nm (log ε)	^1H NMR (solv.), δ	^{13}C NMR (solv.), δ
2a	-	$C_{15}H_{19}NO_2$ 72.71	73.44 7.82	7.80 5.73	5.71	(KBr) 3500-3100 (OH)	273 (3.04) 267 (3.07) 260 (3.01)	($CDCl_3$) 1.55-2.00 (4H, m, 2xCH_2), 2.35-3.00 (5H, m, N(CH_2)2, and OH exchanged with D_2O), 3.30-3.80 (6H, m, -CH-N, O(CH_2)2, and benzylic H), 7.00-7.45 (4H, m, ArH)	($CDCl_3$) (arom. C) 111.06, 110.19, 103.21, 102.31, 99.81, 99.74, (aliph. C) 83.17 (ArCOH), 73.85 (-CH-N), 71.86 (OCH_2)2), 67.18 (benzylic CH), 64.96 (-N(CH_2)2), 53.00, 50.84 (2xCH_2)
2b	96 (Et_2O-PE)	$C_{16}H_{21}NO_2$	74.09 74.26	8.16 8.24	5.40 5.32	(KBr) 3500-3200 (OH)	272.5 (3.00) 266 (3.05) 258.5 (3.02)	($CDCl_3$) 0.95-2.05 (6H, m, 3xCH_2), 2.35-2.60 (2H, m, -N-CH_2), 2.80-3.15 (3H, m, -N-CH, N-CH_2), 3.60-3.85 (6H, m, O(CH_2)2, benzylic H and OH exchanged with D_2O), 7.00-7.35 (4H, m, ArH)	($CDCl_3$) (arom. C) 147.72, 145.72, 128.85, 127.42, 122.95, (aliph. C) 79.54 (ArCOH), 67.56 (OCH_2)2), 66.66 (-CH-N), 52.52 (benzylic CH), 50.11 (-N(CH_2)2), 21.90, 17.37, 15.30 (3xCH_2)
2c	120 (PE)	$C_{17}H_{23}NO_2$	74.68 74.31	8.48 8.75	5.12 5.39	(KBr) 3600-3200 (OH)	274.5 (3.30) 268 (3.29) 262 (3.13)	($CDCl_3$) 1.18-1.55 (3H, m, CH_2-CH), 1.80-2.05 (4H, m, 2xCH_2), 2.10-2.25 (1H, m, -CH) 2.40-2.60 (3H, 1xCH_2, and N-CH-), 2.62-2.85 (1H, m, OH exchanged with D_2O), 2.90-3.05 (2H, m, 1xCH_2), 3.20-3.35 (1H, m, benzylic H), 3.65-3.80 (4H, m, O(CH_2)2), 7.45 (4H, m, ArH)	($CDCl_3$) (arom. C) 148.97, 146.01, 129.07, 127.24, 122.62, 122.43, (aliph. C) 86.19 (ArCOH), 68.38 (-CH-N), 67.93 (OCH_2)2), 59.46 (benzylic CH), 51.50 (-N(CH_2)2), 31.15, 30.28, 27.59, 22.95 (4xCH_2)
2d	151 (Et_2O-PE)	$C_{17}H_{23}NO_2$	74.68 75.16	8.48 8.55	5.12 5.09	(KBr) 3600-3200 (OH)	272.5 (3.23) 266 (3.24) 20 (3.12)	($CDCl_3$) 0.75-1.25 (3H, m, -CH, and -CH_2), 1.55-2.00 (4H, m, 2xCH_2), 2.10-2.25 (1H, N-CH-), 2.45-3.05 (5H, m, -CH-, and 2xCH_2), 3.65-4.05 (5H, m, O(CH_2)2, benzylic H), 5.65-6.10 (1H, OH exchanged with D_2O), 7.00-7.45 (4H, m, ArH)	($CDCl_3$) (arom. C) 148.47, 146.19, 128.69, 127.21, 123.13, 122.82 (aliph. C) 83.15 (ArCOH), 70.32 (-CH-N), 67.38, 67.23 (OCH_2)2), 55.41 (benzylic CH), 49.03 (-N(CH_2)2), 29.40, 27.27, 24.48 23.39 (4xCH_2)
2e	208 (EtOAc-Et_2O)	$C_{17}H_{23}NO$	79.34 78.58	8.95 8.79	5.45 5.21	(KBr) 3500-3100 (OH)	273 (3.17) 265.5 (3.20) 260 (3.06)	(CD_3OD) 1.20-2.20 (12H, m, 6xCH_2), 2.60-3.00 (5H, m, -N(CH_2)2, and -CH-N), 3.20-3.60 (2H, m, benzylic H and OH, exchanged with D_2O), 6.90-7.30 (4H, m, ArH)	(CD_3OD) (arom. C) 148.38, 147.34, 131.31, 129.31, 125.18, 123.78, (aliph. C) 84.92(ArCOH), 71.58 (-CH-N), 61.45 (benzylic CH), 55.32 (-N(CH_2)2), 33.18, 29.71, 29.53, 28.72 (4xCH_2)
2h	142 (EtOAc-PE)	$C_{16}H_{23}NO$	78.32 77.76	9.45 9.71	5.70 5.48	(KBr) 3600-2500 (NH) 3300 (OH)	272 (3.12) 266 (3.28) 260 (3.12)	($CDCl_3$) 0.92-2.40 (16H, m with d.d at 1.08, CH-(CH_3)2, OH and NH exchanged with D_2O and 4xCH_2), 2.78-3.06 (2H, m, C<u>H</u>-(CH_3)2, and C<u>H</u>NHiPr), 3.36-3.68 (1H, m, benzylic H), 7.00-7.34 (4H, m, ArH)	($CDCl_3$) (arom. C) 148.92, 146.29, 128.95, 127.31, 121.48, (aliph. C) 84.19 (C-OH), 60.27 (CH-NH), 56.98 (benzylic -CH), 46.69 (<u>C</u>H(CH_3)2), 24.35 (CH(C<u>H</u>3)2), 31.00, 29.71, 26.97, 22.96 (4xCH_2)
2g	94 (CH_3COCH_3-PE)	$C_{13}H_{17}NO_2$	71.23 71.55	7.76 7.05	6.39 6.35	(KBr) 3700-2300 (OH, NH_2)	276 (3.29) 270 (3.11)	($CDCl_3$) 0.96-2.47 (8H, m, 4xCH_2), 2.75-3.14 (1H, m, -C<u>H</u>-NH_2), 3.14-3.67 (1H, m, benzylic H), 4.20-4.73 (4H, m, 2xOH and NH_2, exchanged with D_2O), 6.43-7.36 (4H, m, ArH)	[a](D_2O) (arom. C) 149.89, 148.13, 133.12, 130.18, 115.92, 115.00, (aliph. C) 81.10 (ArCOH) 58.81 and 58.71 (benzylic CH and -CH-NH_2), 30.80, 29.00, 28.59, 26.92 (4xCH_2)
3	109 (EtOAc-PE)	$C_{14}H_{15}NO_2$	73.34 72.32	6.59 6.33	6.10 6.09	(KBr) 3400-3000 (NH) 1750 (C=O)	273.5 (3.33) 267 (3.34) 259.5 (3.15)	($CDCl_3$) 1.20-2.50 (8H, m, 4xCH_2), 3.95-4.30 (2H, m, benzylic H and -CH-NH-), 8.75 (1H, m, -NH-), 7.20-7.70 (4H, m, ArH)	
4	66 (Et_2O-PE)	$C_{15}H_{17}NO$	79.26 78.96	7.53 7.21	6.16 5.91	(KBr) 1680 (-C=N-)	275 (3.38) 267 (3.40) 262 (3.25)	($CDCl_3$) 1.00-2.20 (11H, m, 4xCH_2 with s at 2.00, CH_3), 3.80-4.42 (2H, m, benzylic H and -CH-N=), 7.00-7.40 (4H, m, ArH)	($CDCl_3$) 165.11 (-C=N-) (arom. C) 145.61, 141.17 128.27, 127.50, 125.65, 123.96, (aliph. C) 95.38 (-C-O-), 77.71 (-CH-N=), 49.38 (benzylic C), 31.27, 30.55, 21.45, 20.31 (4xCH_2), 14.59 (CH_3)
5	96 (Et_2O-PE)	$C_{17}H_{23}NO_2$	74.68 74.59	8.48 8.63	5.12 5.10	(KBr) 3450-3200 (OH)	272.5 (3.16) 265.5 (3.19) 259.5 (3.04)	($CDCl_3$) 1.40-2.55 (10H, m, 5xCH_2), 2.60-2.92 (4H, m, N(CH_2)2), 3.45-3.80 (4H, m, O(CH_2)2), 5.05-5.40 (1H, m, OH exchanged with D_2O), 7.00-7.35 (4H, m, ArH)	(C_6D_6) (arom. C) 151.34, 145.18, 129.18, 124.74, 123.40, (aliph. C) 84.26 (ArCOH), 79.53 (ArCN), 67.49 (O(CH_2)2), 48.05 (N(CH_2)2), 36.16 32.13, 26.96, 25.57, 25.41 (5xCH_2).

TLC (EtOAc–hexane, 30%), the mixture was quenched by the cautious addition of EtOAc. Ether and water were added, the layers were separated, the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was chromatographed on silica gel chromatography (eluent: 50% EtOAc/petroleum ether) to yield the amino-alcohol **6a** (370 mg; 1.52 mM; 93%): mp 148°C (PE); IR (KBr): ν 3300–3000 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 1.55–2.05 (4 H, m, 2 CH_2), 2.40–3.10 (6 H, m, benzylic CH_2 and $-N(CH_2)_2$), 3.55–4.05 (5 H, m, –CH–N and $O(CH_2)_2$), 4.90 (1 H, d, J = 1.75 Hz, –CHOH), 5.10–5.30 (1 H, m, OH, exchanged with D_2O), 6.95–7.55 (4 H, m, ArH). Anal calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; N, 5.66. Found: C, 72.72; H, 8.68; N, 5.56.

A solution of the amino-alcohol **2d** (500 mg; 2.02 mM) in 10 ml of HMPA was added to a suspension of $NaNH_2$ (120 mg; 3 mM) in 10 ml of HMPA at room temperature. After 15 min, the reaction was complete and the mixture was poured into ice/water and extracted with ether. The organic layer was washed successively with water and brine, then dried ($MgSO_4$) and evaporated *in vacuo*. A rapid column chromatography over silica gel (40% EtOAc in petroleum ether) afforded the unstable amino ketone **12b** (320 mg; 1.3 mM; 64%) which was subsequently reduced.

The above amino ketone dissolved in ether (20 ml) was reduced with $LiAlH_4$ (100 mg; 2.6 mM) at ambient temperature and worked up in a manner similar to that described to yield the amino-alcohol **6b** (300 mg; 1.22 mM; 94%): mp 120°C (PE); IR ν 3400–3100 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 1.05–1.95 (8 H, m, 4 CH_2), 2.35–2.95 (7 H, m, $-N(CH_2)_2$ and benzylic CH_2), 3.55–3.90 (4 H, t, $O(CH_2)_2$), 4.60–4.95 (1 H, m, OH, exchanged with D_2O), 5.10 (1 H, d, J = 1.5 Hz, –CHOH), 7.05–7.50 (4 H, m, ArH). Anal calcd for $C_{17}H_{25}NO_2$: C, 74.14; H, 9.15; N, 5.10. Found: C, 73.72; H, 9.25; N, 5.15.

Pharmacology

Guinea pig isolated trachea

Tracheal spirals containing 2–4 cartilaginous rings were obtained from male guinea pigs (250–350 g) that had been anaesthetized with urethane (1.25 g·kg^{-1}) and were equilibrated under an initial tension of 1.2 g in a physiological solution maintained at 37°C and gassed with O_2 + CO_2 (95/5). Tension was measured isometrically with a Ugo Basil strain gauge and was displayed on a Ugo Basil channel pen recorder. The initial tension ensured that after a 1.5 h equilibration period the resting tension was between 0.5–1 g.

The composition of the physiological solution was (mM): NaCl 139.2; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 0.49; $NaHCO_3$ 11.3; Na_2HPO_4 and glucose 5.5).

In all experiments, tracheal spirals were first contracted to maximal tension with acetylcholine 3 x 10^{-3} M. After 1 h rest, with washing every 15 min, the preparations were contracted to 70–90% of maximal tension with acetylcholine 2 x 10^{-5} M, histamine 2 x 10^{-5} M or KCl 10 mM and, after stabilization, the effects of theophylline or the compounds were tested curatively by adding the drugs to the bath in cumulative concentrations.

The results are expressed as percent inhibition of the tension induced by the spasmogenic agents. pD_2 represented the –log molar concentration of the reagent that caused 50% of maximal effect.

Experiments performed to determine the adenosine-antagonist effect of compound **1b** were carried out according to Advenier *et al* [9]. Tracheal strips were pretreated with dipyridamole 10^{-5} M + theophylline 10^{-5} to 10^{-4} M or compound **1b** (10^{-5} and 3 x 10^{-5} M) before addition of histamine 2 x 10^{-5} M; cumulative concentration responses of adenosine were obtained by increasing the concentration of adenosine at 5–10 min intervals in logarithmic increments. pA_2 values were determined according to Arunlakshana and Schild [10].

Anaesthetized guinea pigs

Male guinea pigs weighing from 0.4–0.6 kg were anaesthetized with urethane (1.25 g·kg^{-1}, ip). A cannula was inserted into the trachea and the animals were allowed to breath spontaneously. Pulmonary airway resistance (R_{aw}) was determined according to the methods of Amdur and Mead [11]. Transpulmonary pressure was measured by needle pleural puncture, and airflow and tidal volume by plethysmography. All values were continuously recorded on a 7700 Hewlett–Packard recorder.

One dose of histamine (25 μg/kg) or ACh (60 μg/kg) was injected on 2 successive occasions (control response), then repeated after intravenous administration of compound **1b** or theophylline in cumulative doses of 1, 3, 30 and 50 mg/kg.

The effects of compound **1b** and theophylline were expressed as % inhibition of control bronchoconstrictor responses. All values are expressed as mean ± SEM.

Drugs

The drugs used were: adenosine (Merck, Darmstadt), histamine (Sigman, St Louis, USA), KCl (Prolabo, Paris), acetylcholine (Pharmacie Centrale des Hôpitaux, Paris), dipyridamole (Boehringer–Ingelheim, Reims), theophylline sodium anisate (Bruneau, Paris). Theophylline was used as proprietary injectable solution (Theophylline Bruneau); dipyridamole was dissolved daily in ethanol and the solution was further diluted with Krebs solution.

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