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New Calcium Antagonists: Synthesis, X-ray Analysis, and Smooth Muscle Relaxing Effect of 3-[*O*-(Benzyl-substituted)-oximino-ethers]-hexahydroazepin-2,3-diones

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Abstract—A series of new *Z* and *E* 3-[*O*-(benzyl-substituted)-oximino-ether]-hexahydroazepin-2,3-diones was prepared from the corresponding hexahydroazepin-2,3-diones and examined as smooth muscle relaxants. *E* and *Z* structures were assigned by NMR analysis and confirmed for **16** (*E* and *Z*) by an X-ray diffraction using synchrotron radiations. The nitrobenzyl derivative **16** was the most potent in vitro as relaxant of rat trachea precontracted with acetylcholine. The *E* isomer **16b** was more potent than the *Z* isomer **16a**. *E* isomer **16b** is more potent than aminophylline to relax both rat trachea and human bronchus.This derivative acts mainly by inhibiting cellular influx of extracellular calcium since it inhibits potently and dose-dependently the contractions of rat trachea to high concentrations of KCl and to $CaCl_2$ in a depolarizing medium. It appears to act weakly by inducing cGMP and cAMP synthesis. Moreover, its relaxing activity is not related to an inhibition of phosphodiesterases, to opening of potassium channels or to induction of prostaglandin synthesis. Therefore, **16b** appears to work mainly as a potent calcium antagonist.

Introduction

It is well documented that various amide or lactame derivatives are endowed with actions on the central nervous system via a stimulation of various ion channels.[1] In a previous work we have shown that oximino-ether derivatives exhibited marked antinociceptive and anticonvulsivant activities;[2] therefore, we became interested in the preparation of new compounds combining within a single structure an oximino-ether function and a lactame ring and more precisely the scarcely examined caprolactame ring.The biological activity of these compounds on airway smooth muscles, which are richly endowed with K^+ and Ca^{++} channels were evaluated. This report describes the preparation and X-ray analysis of these new oximino-caprolactames with attribution of their *Z* and *E* configurations, evaluation of their spasmolytic activity on rat trachea and human bronchus and examination of their mechanism of action.

Chemistry

Our strategy for the synthesis of target compounds **11**–**18** (Scheme 1) was based on the construction of the hexahydroazepin-2,3-dione skeleton **4**, followed by attachment of the substituted *O*-hydroxylamines **5**–**10**. Duong et al.[3] obtained compound **4** by coupling hydrazoic acid and 2-[2-(1-cyclohexanone)]-1,3-dioxolane with polyphosphoric acid. The resulting instable intermediate, gave **4** after a Schmidt transposition, but with a very poor yield (2%) and the hydrolysis of enamine was more conveniently accomplished on a silica gel column to provide pure oxolactam in high yield, but only with hexa or pentacyclic oxolactam.[4] We have prepared this compound by hydrolysis of the ene-morpholine **3** in acidic medium. This route afforded hexahydroazepin-2,3-dione **4** with 30% yield. Various methods are available for the preparation of α,α-dichlorocaprolactam **2**.[5,6] The use of phosphorus pentachloride

Key words: Hexahydroazepin-2,3-diones; synthesis; X-ray; calcium antagonist; muscle relaxing.

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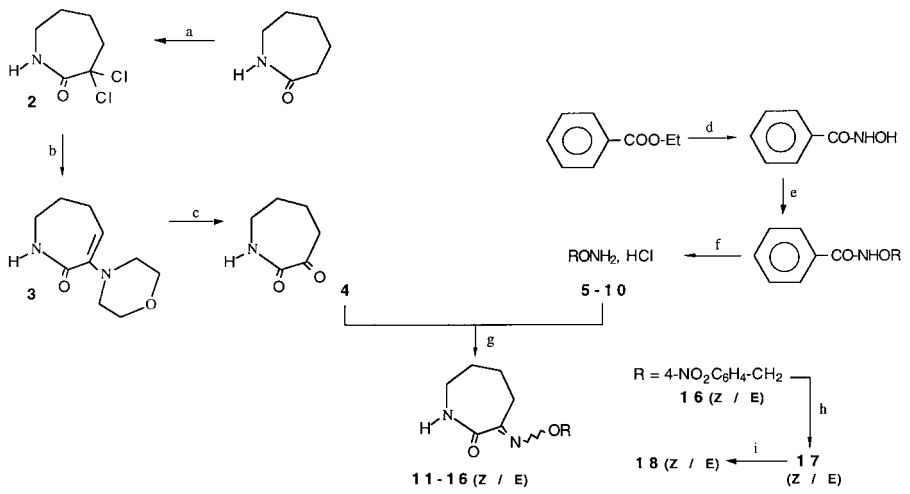

Scheme 1. Synthesis of oximino caprolactame derivatives. (a) PCl_5; (b) morpholine; (c) H_2O/H^+; (d) NH_2OH; (e) KOH/EtOH, R-Br; (f) H_2O/H^+; (g) pyridine; (h) H_2/Pd; (i) CH_3-SO_2-Cl.

without solvent allowed us to notably increase the reported yields. Treatment of compound **2** with morpholine led to the ene-morpholine **3**.[7,8] Compounds **5**–**10** were then prepared according to Garoufalias et al.[9] (Scheme 1), whereas compounds **11**–**16** (Table 1) were obtained from **5**–**10** by reaction with **4** in refluxing ethanol containing 5–10% of pyridine. Reduction of the nitro derivative **16** using H_2/Pd gave the amino derivative **17**, which was finally treated by CH_3SO_2Cl to obtain the sulfonamide **18**. Oximino-ethers **11**–**18** were isolated as mixtures of *Z* and *E* isomers in which the *E* isomer predominated. Isomerisation of **16a** to **16b** occurred when **16a** (*Z*) was left aside for some days into ethylacetate, at room temperature. Ratio of isomers were assessed by NMR spectroscopy on the basis of the chemical shifts observed for protons on carbon adjacent to the oxime double bond.[10] In accordance with assignments reported in the literature for several oximes and alkylated oximes,[11] *Z* isomers are more downfield than their *E* counterparts. In order to verify these assignments, an unambiguous structure determination of the two *Z* and *E* isomers, **16a** and **16b** of 3-[*O*-(4-nitrobenzyl)-oximino-ether]-hexahydroazepin-2,3-diones was finally accomplished through X-ray diffraction. The results obtained are in full agreement with NMR data.

Table 1. Physiochemical data

Compd	R	mp (°C)	Yield%	Formula (CNH)[c]
5	CH_3[a]			
6	$C_6H_5CH_2$	229–231[b]	55	$C_7H_{10}NOCl$
7	4-$CNC_6H_4CH_2$	197–199	25	$C_8H_9N_2OCl$
8	2-$NO_2C_6H_4CH_2$	134–136	30	$C_7H_9N_2O_3Cl$
9	3-$NO2C_6H_4CH_2$	143–145	30	$C_7H_9N_2O_3Cl$
10	4-$NO_2C_6H_4CH_2$	216–218[b]	35	$C_7H_9N_2O_3Cl$
11a	CH_3(*Z*)	137–139	22	$C_7H_{12}N_2O_2$
11b	CH_3(*E*)	135–137	78	$C_7H_{12}N_2O_2$
12a	$C_6H_5CH_2$(*Z*)	69–71	10	$C_{13}H_{16}N_2O_2$
12b	$C_6H_5CH_2$(*E*)	74–76	90	$C_{13}H_{16}N_2O_2$
13a	4-$CNC_6H_4CH_2$(*Z*)	141–143	20	$C_{14}H_{15}N_3O_2$
13b	4-$CNC_6H_4CH_2$(*E*)	139–141	80	$C_{14}H_{15}N_3O_2$
14a	2-$NO_2C_6H_4CH_2$(*Z*)	124–126	35	$C_{13}H_{15}N_3O_4$
14b	2-$NO_2C_6H_4CH_2$(*E*)	119–121	75	$C_{13}H_{15}N_3O_4$
15a	3-$NO_2C_6H_4CH_2$(*Z*)	126–128	30	$C_{13}H_{15}N_3O_4$
15b	3-$NO_2C_6H_4CH_2$(*E*)	107–109	70	$C_{13}H_{15}N_3O_4$
16a	4-$NO_2C_6H_4CH_2$(*Z*)	120–122	40	$C_{13}H_{15}N_3O_4$
16b	4-$NO_2C_6H_4CH_2$(*E*)	103–105	60	$C_{13}H_{15}N_3O_4$
17a	4-$NH_2C_6H_4CH_2$(*Z*)	163–165	10	$C_{13}H_{17}N_3O_2$
17b	4-$NH_2C_6H_4CH_2$(*E*)	154–156	90	$C_{13}H_{17}N_3O_2$
18a	4-$CH_3SO_2NHC_6H_4CH_2$(*Z*)	Oil	20	$C_{14}H_{19}N_3O_4S$
18b	4-$CH_3SO_2NHC_6H_4CH_2$(*E*)	Oil	80	$C_{14}H_{19}N_3O_4S$

[a] Aldrich compd.
[b] Literature values: **6**, 230°C;[19] **10**, 217°C.[19]
[c] Symbols in parentheses refer to elements analyzed.

Results and Discussion

X-ray crystallographic study of 16a and 16b

The two isomers, **16a** (*Z*) and **16b** (*E*), were selected for X-ray crystallographic study. Their molecular structures are presented showing the atoms labelling (Figs 1 and 2). The configuration of the C8–N2 double bond (1.275 (6) Å) is *syn* (*Z*) for **16a** and *anti* (*E*) for **16b**, with distances O3–C9 = 3.614 (9) Å for the *E* isomer and 2.569 Å for the *Z* isomer. The caprolactame ring has a chair or pseudo-chair conformation as determined on the one hand by the distances of atoms C9, C11 and C12 to the plane P1 defined by C8C13C10N3, on the other hand by the distances of atoms C12, C9 and N3 to the plane defined by C8H13AC11C10 (H13A is above H13B).

The molecules are held together by hydrogen bonds between H3...O4 (d = 2.119 (9)Å) for *E* isomer and by H3...N2 (d = 2.180 (14)Å) for *Z* isomer.

Pharmacology

Relaxant effects of the oximino-caprolactame derivatives on the rat trachea were evaluated. The most potent oximino-caprolactame derivatives were found to be compounds **16** (**16a** + **16b**), **16b** being more potent than **16** and **16a** (Fig. 3). The concentration of **16b** that caused a relaxation equal to 50% of the maximal relaxation induced by aminophylline (IC_{50}) was 36.1×10^{-6} M and the maximal relaxation at the highest concentration (3×10^{-4} M) was 88 ± 7.5%. This compound is about twofold more potent than **16** ($IC_{50} = 68.1\times10^{-6}$ M) and more than 10-fold more potent than **16a** ($IC_{50} > 3\times10^{-4}$ M). The activities of oximino-caprolactame derivatives are shown in Table 2.

The comparison with classical relaxing drugs of the effect of **16b** has been performed on the rat trachea and the human bronchus (Fig. 4). On the rat trachea, comparison of the IC_{50} indicates that **16b** appears as potent as verapamil, is about 14-fold less potent than cromakalim and 19-fold more potent than aminophylline. In addition, the comparison with isoprenaline, due to its maximal effect inferior to 50% of the maximal relaxation with aminophylline,[12] can be done by taking into account the IC_{25} of isoprenaline (5.35×10^{-7} M) and the IC_{25} of **16b** (8.71×10^{-6} M). It shows that the oximino-caprolactame compound is about 16-fold less potent than isoprenaline but has a much higher efficacy in rat trachea (Fig. 4). In human bronchus, by the same way of comparison, **16b** appears fourfold more potent than aminophylline but fivefold and 14-fold less potent than verapamil and cromakalim and also 110- and 750-fold less potent than salbutamol and isoprenaline, respectively (Fig. 4, Table 3). Compound **16b** did not act by opening ATP-potassium channels, like cromakalim, since its relaxant effect is not inhibited by glibenclamide (10^{-5} M, $n = 5$), a potent and selective blocker of this type of potassium channels. It did not appear to act by opening other types of potassium channels that may be involved in the relaxation of airway smooth muscle since

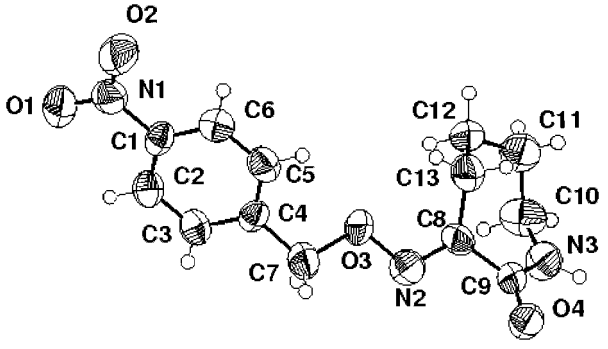

Figure 1. 16b (*E*) crystallographic molecular structure with atoms labelling.

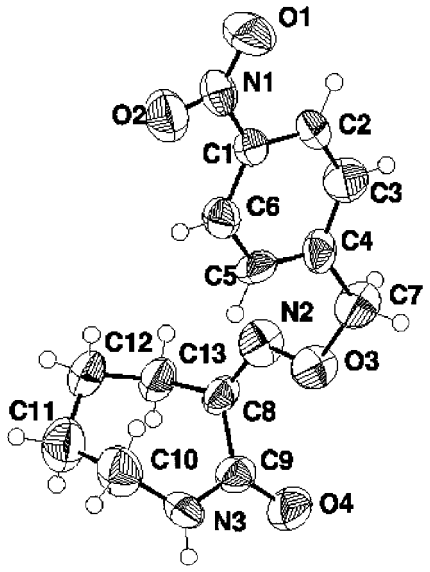

Figure 2. 16a (*Z*) crystallographic molecular structure with atoms labelling.

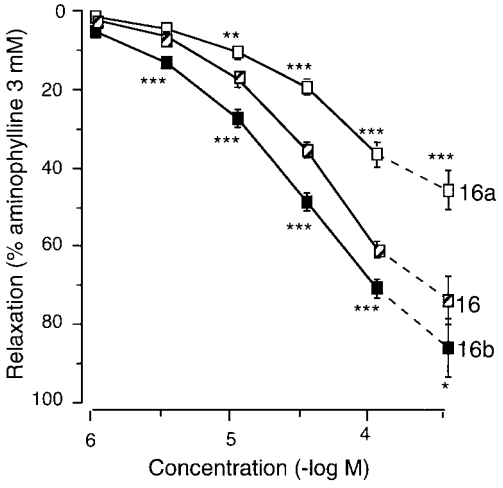

Figure 3. Concentration–relaxation curves to **16** ($n = 23$), **16a** ($n = 7$) and **16b** ($n = 17$) in rat trachea precontracted with acetylcholine. Values are mean ± S.E.M. calculated on the number of experiments indicated in parentheses excepted for the data at 3×10^{-4} M concentration, for which the mean ± S.E.M. have been calculated on five to seven experiments. Significant differences from **16** are $^{*}p < 0.05$; $^{**}p < 00.1$ $^{***}p < 000.1$

Table 2. Relaxant effects of the oximino-caprolactame derivatives on the rat trachea

Compds	Rat trachea			
	n	IC_{50}[a] (95% CI)[b]	I.A.	R.A.
16b	17	36.1 (14.2–92.3)	0.70 ± 0.02	100
16a	7	> 300	0.35 ± 0.04	50
11b	5	–	0	0
12b	5	> 300	0.025 ± 0.04	3.5
13b	4	> 300	0.30 ± 0.16	43
14b	4	–	0	0
15b	4	> 300	0.033 ± 0.05	5
18b	4	–	0	0

[a] = values × 10^{-6} M.

[b] IC_{50} (concentration inducing 50% of the maximal relaxation induced by 3 mM aminophylline) with 95% confidence limits and I.A. (intrinsic activity) defined as the effect at 10^{-4} M, relative to a maximal relaxation to aminophylline 3 mM. The relative activity (R.A.) is the ratio between the I.A. of each oximino-caprolactame tested and that of **1**–**6b** defined as 100.

the relaxation to **16b** was not altered in KCl-enriched medium ($n = 4$) or in the presence of a non-selective blocker of potassium channels (TEA, 10^{-3} M ($n = 4$) and 10^{-2} M ($n = 3$)) (data not shown). The effect of **16b** on the KCl- and $CaCl_2$-induced contractions on rat trachea at baseline tone (not contracted with acetylcholine) is shown in Figure 5. This compound inhibited effectively and dose-dependently these contractions of the airway smooth muscle that are directly caused by the cellular influx of extracellular calcium. **16b**, like calcium antagonists,[13] displaced concentration–response curves to the right with depression of the maximal responses. The comparison of **16b** with verapamil (Fig. 6), a classical calcium antagonist acting by blocking the voltage-sensitive calcium channels, shows that the oximino-caprolactame compound is 10- to 30-fold less potent than verapamil. Indomethacin (10^{-6} M, $n = 6$) did not inhibit the relaxation to **16b** indicating that **16b** did not act through the induction of prostaglandin synthesis. The relaxation induced by **16b** is however potentiated by the selective inhibitor of the cGMP-phosphodiesterase (type V), zaprinast, at 10^{-5} M ($n = 7$) but not at 10^{-6} M ($n = 6$) (Fig. 7). The potentiation by zaprinast of the relaxation to **16b** (2.6-fold shift to the left of the concentration response curve to **16b**) is, by far, weaker than the potentiation by zaprinast of the relaxation to SNP, a classical activator of the guanylyl cyclase (data not shown). These data suggest that **16b** may weakly promote the synthesis of cGMP. However, the concentration–relaxation curve to **16b** is not significantly inhibited by three inhibitors of the soluble guanylyl cyclase, methylene blue (10^{-4} M, $n = 12$), dipyridamole (10^{-6} M, $n = 5$) and *N*-methylhydroxylamine (10^{-4} M, $n = 6$), indicating further that the synthesis of cGMP exerts a minor role in **16b** relaxation pathway. The relaxation to **16b** is also weakly but significantly potentiated by rolipram (10^{-6} M, $n = 16$), a selective inhibitor of the cAMP-phosphodiesterase (type IV) as shown by the 1.5-fold shift to the left of the concentration-response curve to **16b** (Fig. 7). This borderline effect is not due to activation of β-adrenergic receptors with subsequent synthesis of cAMP since the response to **16b** was not inhibited by propranolol (10^{-6} M, $n = 7$), a potent β_1- and β_2-receptor antagonist. The relaxation to **16b** is also not potentiated by siguazodan (10^{-6} M, $n = 4$; 10^{-5} M, $n = 6$), a selective inhibitor of the type III phosphodiesterase, which is also a phosphodiesterase important for the regulation of cAMP breakdown in airway smooth muscle, notably in murine airways.[14] These results, indicate that, as shown above for cGMP, AMPc do not clearly participate in the mechanism of the relaxation induced by **16b**. Collectively, these results suggest that **16b** may weakly stimulate cGMP and, to a lesser extent, cAMP syntheses but the increase in cGMP and in cAMP do not explain the relaxant effect of this compound.

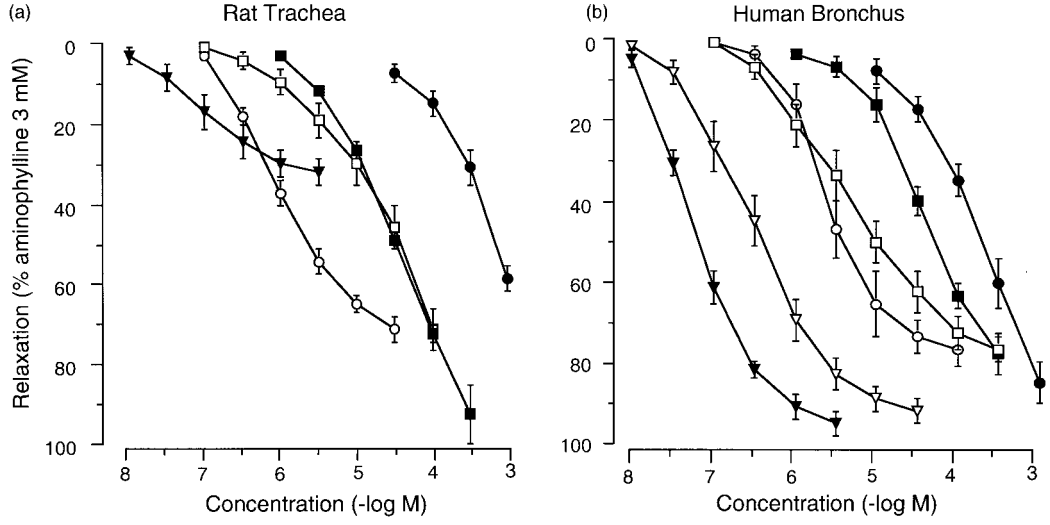

Figure 4. Concentration–relaxation curves to **16b** (■) and reference relaxant compounds:isoprenaline (▼), cromakalim (○), salbutamol (▽), (verapamil (□) and aminophylline (●) in rat trachea (left panel) and human bronchus (right panel) precontracted with acetylcholine. Values are mean ± S.E.M. of the number of experiments shown in Table 1.

Table 3. Effect of **16b** and reference relaxant compounds in rat trachea and human bronchus precontracted with acetylcholine

Compds	Rat trachea			Human bronchus		
	n	IC_{50}[a] (95% CI)[b]	I.A.	*n*	IC_{50}[a] (95% CI)[b]	I.A.
16b	17	36.1 (14.2–92.3)	0.70 ± 0.02	7	52.3 (11.5–220)	0.61 ± 0.03
Isoprenaline	9	ND	0.31 ± 0.03	6	0.07 (0.03–0.16)	0.93 ± 0.02
Salbutamol	NT	—	—	10	0.47 (0.16–1.10)	0.89 ± 0.03
Aminophylline	8	676 (323–1412)	0.15 ± 0.04	5		
Cromakalim	11	2.5 (1.5–4.1)	0.67 ± 0.03	5	3.8 (0.8–18.2)	0.75 ± 0.06
Verapamil	9	40.7 (13.2–128)	0.68 ± 0.05	5	10.3 (2.4–42.2)	0.72 ± 0.08

[a] = values × 10^{-6} M; NT = not tested; ND = not determined.
[b] IC_{50} (concentration inducing 50% of the maximal relaxation induced by 3 mM aminophylline) with 95% confidence limits and I.A. (intrinsic activity) defined as the effect at 10^{-4} M, or the maximal effect, if this occurred at a lower concentration, relative to a maximal relaxation to aminophylline 3 mM.

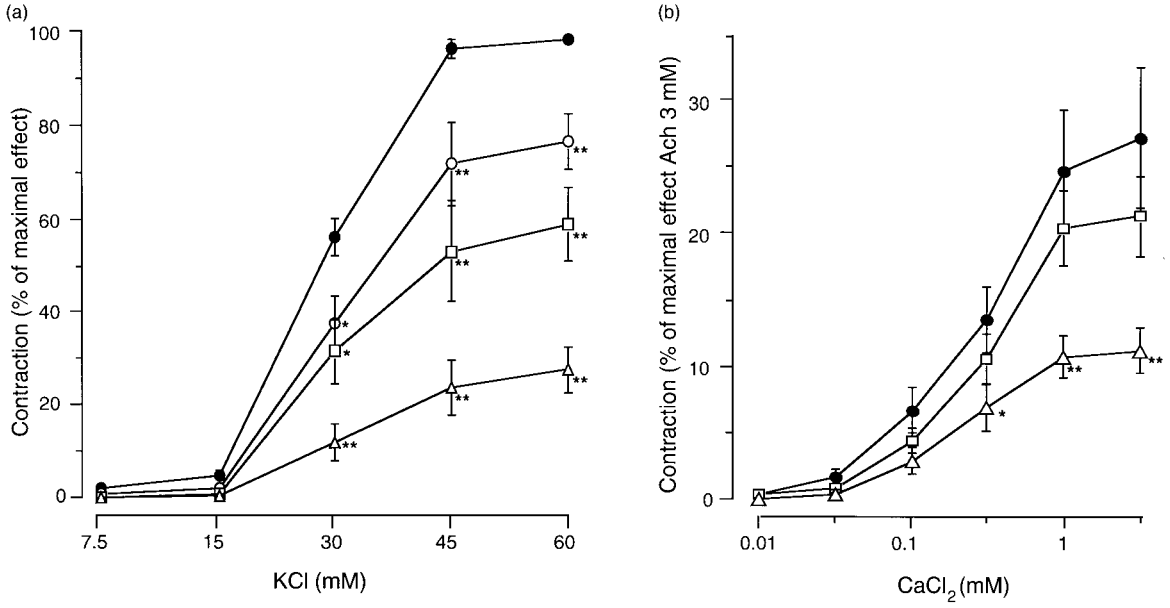

Figure 5. Effect of **16b** in the KCI- and $CaCl_2$-induced contractions in rat trachea at basal tone. Concentration–response curves to KCI or $CaCl_2$ were performed after 20 min incubation with **16b** (10^{-6} M: ○; 10^{-5} □; 10 M^{-4} M, △) or the solvent (control; ●). Values are mean ± of five to 12 experiments. Significant differences from control are: *$p < 0.005$; **$p < 0.01$.

Finally, **16b** did not act, like theophylline, as a phosphodiesterase inhibitor because this compound did not potentiate the concentration–relaxation curves to activators of the adenylyl-cyclase, isoprenaline ($n = 5$) and forskolin ($n = 6$), as well as to the activator of the guanylyl-cyclase, sodium nitroprusside ($n = 6$) (data not shown).

Taken as a whole, the pharmacological data suggest that **16b** acts mainly as a calcium antagonist on airway smooth muscle. In conclusion, we have observed that 3-[*O*-(benzyl-substituted)-oximino-ether]-hexahydroazepin-2,3-diones may represent a new and valuable class of calcium antagonists.

Experimental

Chemistry

Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. Infra-red spectra were obtained on a Pye-Unicam SP3-100 (Philips) spectrometer. Nuclear magnetic resonance spectra were recorded on an AC 200 FT (Bruker) spectrometer ^{1}H NMR at 200 MHz and ^{13}C NMR at 50 MHz. Chemical shifts are reported in parts per million (δ) downfield relative to tetramethylsilane. Electron ionization (EI) mass spectra were obtained on a Nermag R 10-10C spectrometer. Elemental analyses (C, H, N) were determined by

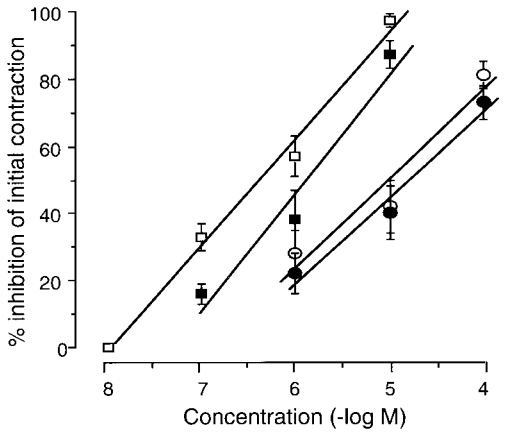

Figure 6. Inhibition by verapamil (squares) and **16b** (circles) of the contractile effects of KCl at 30 mM (open symbols) and at 60 mM (closed symbols) in rat trachea at basal tones. Experiments were performed on groups of six to 12 preparations. Points represent mean with S.E.M. shown by vertical lines.

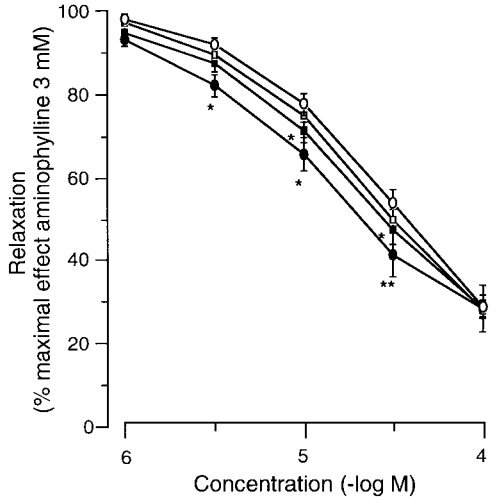

Figure 7. Influence of rolipram (squares) or zaprinast (circles) on the concentration–response curves to **16b** in rat trachea precontracted with acetylcholine. Concentration–response curves to **16b** were performed after 20 min incubation with saline (control; □, ○), rolipram ($n=16$, ■) or zaprinast 10^{-5} M ($n=7$, ●). Values are mean ± S.E.M. Significant differences from control are: $*p<0.05$; $**p<0.01$ (student's test for paired data).

CNRS-Vernaison, France, and were within ±0.4% of the calculated values.

General procedure for the preparation of compounds 5–10

To a suspension of KOH powder (19.6 g, 0.35 mol) in anhydrous pyridine (120 mL) at 0°C, hydroxylamine hydrochloride 13.9 g, 0.2 mol) dissolved in anhydrous pyridine (100 mL) were added. The ethylbenzoate (15.0 g, 0.1 mol) was added keeping the temperature between 0 and 5°C. The resulting mixture was allowed to stir for 6 h at room temperature. The solid formed was filtered, washed several times with water, with 0.01 N HCl and the residue was dissolved in CH_2Cl_2. The organic phase was dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow solid of benzhydroxamic acid. 80% yield; mp 130–132°C; lit.[15] = 131°C. To a solution of this acid (25.07 g, 0.183 mol) in ethanol (28 mL), was added a solution of KOH (10.3 g, 0.184 mol) in water (40 mL) followed by a solution of alkyl bromide in ethanol. The mixture was heated under reflux for 0.75 h. The resulting solution was cooled and the precipitate formed was filtered. The crystals were dissolved in EtOH (125 mL). Concentrated HCl (150 mL) was added to the latter solution, and the mixture was heated under reflux for 0.25 h. Evaporation in vacuo gave a crude residue which was partitioned between chloroform (200 mL) and water (150 mL). The aqueous phase was washed several times with $CHCl_3$ and evaporated to give a solid which was recrystallized from 2 N HCl. Overall yields are low (ca. 30%) because hydroxamic acid decomposes during the alkylation step via the Lossen rearrangement to give aniline. Physical constants are given as an example for compound **7**.

***O*-(4-Cyanobenzyl)-hydroxylamine, HCl (7).** IR (KBr 2%) 3300, 910 cm^{-1}; ^1H NMR (200 MHz, DMSO) δ 5.22 (2H, s), 7.45 (2H, m,), 7.64 (2H, m,); 11.2 (2H, s); ^{13}C NMR (50 MHz, DMSO) 75, 118.6, 128.2, 132.2, 142.0. Anal. ($C_8H_9N_2OCl$) C, H, N.

α,α-Dichlorocaprolactam (2). In a 1-L, three-necked flask fitted with a mechanical stirrer and a reflux condenser, caprolactame (11.3 g, 0.1 mol) and PCl_5 (62.54 g, 0.3 mol) were placed. The mixture was stirred at 90°C during 0.25 h, cooled to 0 C and hydrolyzed with a concentrated aqueous solution of Na_2CO_3. The solid formed was filtered, and washed successively with H_2O, EtOH, and Et_2O. 70% yield; mp 126–128°C [lit.[16] = 126.5°C]

Morpholino-3-ene caprolactame (3). A mixture of anhydrous morpholine (0.23 mol, 20 g) and powdered Drierite was heated under reflux in a flask fitted with a $CaCl_2$ tube. After complete deoxygenation, α,α-dichlorocaprolactam **2** (2 g, 0.01 mol) was added and the mixture was heated at 100°C for 6 h. The excess of morpholine was evaporated in vacuo and the crude residue was partitioned between $CHCl_3$ and H_2O (100 mL each). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Trituration with a minimum of EtOAc afforded **3** as a solid. 45% yield; mp 125–127°C [lit.[7] = 126°C]

Hexahydroazepin-2,3-dione (4). To an ice-cooled solution of enamine **3** (9.8 g, 0.05 mol) in EtOH (50 mL) was added concentrated HCl (15 mL). After stirring for 0.6 h the ethanol was evaporated in vacuo and CH_2Cl_2 (100 mL) was added. The organic phase was washed with aqueous $NaHCO_3$, two times with water, dried over $MgSO_4$, and concentrated under reduced pressure to give **4** as a white solid. 35% yield; mp 67–69°C.

General procedure for the preparation of 3-[*O*-(aryl)-oximinoether]-hexahydroazepin-2,3-diones 11–16. The preparation of **16** is illustrative of the method. To a solution of hexahydroazepine-2,3-dione **4** (1.01 g,

0.008 mol) in absolute EtOH (10 mL) was added 4-nitrobenzylhydroxamine, HCl **10** (2.5 g, 0.008 mol) and pyridine (1 mL). The mixture was heated under reflux for 4 h. The solvent was evaporated in vacuo and water (25 mL) was added. The aqueous solution was extracted by CH_2Cl_2 (3×10 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated. The mixture of isomers was separated by silica gel column chromatography (ethylacetate/cyclohexane, 8/2) to afford the corresponding *Z* and *E* oximino-ethers. Physical constants are given as examples for compounds **11, 12**, and **16**.

3-[*O*-Methyl oximino-ether]-hexahydroazepin-2,3-diones (11). IR (KBr 2%) 3200, 1700, 1610 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) **11a** δ 1.74 (4H, m), 2.40 (2H, dd), 3.17 (2H, td), 3.87 (3H, s), 7.26 (1H, t); **11b** δ 1.77 (4H, m), 2.51 (2H, dd), 3.24 (2H, td), 3.97 (3H, s), 7.49 (1H, t); ^{13}C NMR (50 MHz, CDCl3) δ **11a** 28.6, 29.40, 42.0, 62.2, 156.40 168.9. **11b** 21.4, 24.4, 27.1, 40.0, 62.6, 156.6, 169.0. Anal. ($C_7H_{12}N_2O_2$) C, H, N; MS: (EI,70 eV, I%), *m*/*e*: M^+ 156 (79.6%); 87.0 (100%).

3-[*O*-Benzyl-oximino-ether]-hexahydroazepin-2,3-diones (12). IR: (KBr 2%) 3210, 1710, 1600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) **12a** δ 1.67 (4H, m), 2.41 (2H, dd), 3.10 (2H, td), 5.15 (2H, s), 7.30 (lH, t), 7.40 (5H, m); **12b** δ 1.72 (4H, m), 2.58 (2H, dd), 3.23 (2H, td), 7.35 (2H, s), 7.38 (lH, t); ^{13}C NMR (50 MHz, $CDCl_3$) δ **12a** 28.0, 29.5, 42.8, 76.0, 127.7, 128.2, 128.3, 138.2, 156.9, 168.8; **12b** 21.5, 24.7, 27.1, 24.7, 40.1, 76.9, 127.9, 128.1, 137.0, 157.0, 168.8. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.; MS: (EI,70 eV, I%), *m*/*e*: M^+ 232 (68,15%); 215 (100%).

3-[*O*-(4-Nitrobenzyl-oximino-ether]-hexahydroazepin-2,3-diones (16). IR: (KBr 2%) 3210, 1690, 1620, 920 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) **16a** δ 1.72 (4H, m), 2.40 (2H, dd), 3.18 (2H, td), 5.22 (2H, s), 6.78 (lH, t), 7.50 (2H, m), 8.19 (2H, m); **16b** δ 1.75 (4H, m), 2.60 (2H, dd), 3.22 (2H, td), 5.31 (2H, s), 7.39 (lH, t), 7.50 (2H, m), 8.18 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ **16a** 28.6, 29.4, 42.1, 74.5, 123.5, 127.7, 145.5, 147.5, 156.2, 168.7; **16b** 21.6, 24.8, 27.1, 40.1, 75.5, 123.6, 144.7, 147.5, 158.1, 168.3. Anal. ($C_{13}H_{15}N_3O_4$) C, H, N.

3-[*O*-(4-Aminobenzyl)-oximino-ether]-hexahydroazepin-2,3-diones (17). To a solution of **16** (1 g, 0.0036 mol) in absolute EtOH (20 mL) was added 0.1 g of 5% Pd on charcoal. Reduction of the nitro-derivative, using Pd/H_2 during 12 h under atmospheric pressure, gave after filtration on Celite and evaporation of EtOH in vacuo, compound **17** as a white solid. IR: (KBr 2%) 3300, 3225, 1700 1620, 910 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) **17a** δ 1,70 (4H, m), 2.12 (2H, s), 2.37 (2H, dd), 3.20 (2H, td), 5.01 (2H, s), 6.50 (1H, t), 6.78 (2H, m), 7.15 (2H, m); **17b** δ 1.73 (4H, m), 2.10 (2H, s), 2.55 (2H, dd), 3.23 (2H, td), 5.11 (2H, s), 6.50 (1H, t), 6.78 (2H, m), 7.19 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ **17a** 28.4, 29.3, 29.3, 41.8, 74.9, 115.5, 119.5, 129.0, 156.2, 168.3; **17b** 21.9, 24.5, 27.5, 40.0, 75.3, 115.2, 119.5, 129.1, 156.2, 168.0. Anal. ($C_{13}H_{17}N_3O_2$) C, H, N.

3-[*O*-(4-Methylsulfonylaminobenzyl)-oximino-ether]-hexahydro-azepin-2,3-diones (18). To a solution of 3-[*O*-(4-aminobenzyl)-oximino-ether]-hexahydroazepin-2,3-diones **17** (1 g, 0.004 mol) in $CHCl_3$ (30 mL) were added CH_3SO_2Cl (0.92 g, 0.008 mol) and triethylamine (0.19 g, 0.0032 mol). The mixture was heated under reflux, during 30 min, in a flask fitted with a $CaCl_2$ tube, then poured in 100 mL of ice-water. The organic phase was dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by silical gel column chromatography and eluted with ethylacetate to give compound **18** as an oil. IR: (KBr 2%) 3250, 1307 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) **18a** δ 1.72 (4H, m), 2.40 (2H, dd), 2.80 (3H, s), 3.16 (2H, td), 5.20 (2H, s), 6.76 (1H, t), 7.52 (2H, m), 8.00 (2H, m), 8.62 (1H, s); **18b** δ 1.74 (4H, m), 2.62 (2H), 2.81 (3H, s), 3.20 (2H, td), 5.25 (2H, s), 6.90 (lH, t), 7.60 (2H, m), 8.20 (2H, m), 8.61 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ **18a** 28.5, 29.4, 29.4, 42.0, 44.0, 75.0, 118.6, 122.5, 128.5, 139.4, 156.2, 168.6; **18b** 21.5, 24.7 27.1, 40.1 44.4, 75.6, 118.5, 122.3, 128.5, 139.3, 156.4, 168.3; Anal. ($C_{14}H_{19}N_3O_4S$) C, H, N.

X-ray analysis for 16a and 16b

A *syn* form isomer, **16a**, and an *anti* form one **16b**, were selected for X-ray crystallographic studies. Crystal data are listed in Table 4.

16b: The single crystals for X-ray analysis recrystallized from ethylacetate. A computer-controlled Enraf–Nonius CAD-4 diffractometer with CuK radiation and an incident beam graphite monochromator were used for X-ray data collection. Structure determination was obtained by direct methods using SHELX86[17] and SHELX93.[18] All the non-hydrogen, 20 atoms, were observed in an *E* map. **16a**: The single crystals for X-ray analysis recrystallized from ethylacetate. Due to the very small size of a single crystal of *Z* isomer, 0.14×0.02×0.01 mm, the crystal structure solution of this isomer was less straightforward. The data collection of this sample was performed by using the X-ray synchrotron radiation; the wavelength is 0.619 Å. In addition to its small size and the instability of the crystal under X-ray radiation only 739 reflections could be measured. The structure of *Z* isomer was solved by direct method as above and difference Fourier syntheses. Atoms distances to plane P1 and P2:

		E	*Z*
to P1	C9	0.214 (16) Å	0.231 (18) Å
	C11	0.300 (25) Å	0.369 (19) Å
	C12	*−0.160 (23) Å*	*−0.055 (18) Å*
to P2	C12	*−1.268 (11) Å*	*−1.202 (09) Å*
	C9	1.153 (12) Å	1.144 (12) Å
	N3	1.351 (11) Å	1.362 (16) Å

Pharmacology

Rat isolated trachea. Male rats (250–350 g) were killed by intraperitoneal injection of 1 mL pentobarbital (6%). The tracheas were excised from the animals and placed in Krebs solution (composition in mM: NaCl 118, KCl 5.4, $CaCl_2$ 2.5, KH_2PO_4 0.6, $NaHCO_3$ 25 and glucose

Table 4. Crystal data for **16b** (*E*) and **16a** (*Z*): **16** is the 3-[*O*-(4-nitrobenzyl-oximino-ether]-hexahydroazepin-2,3-diones

	16b (*E*)	**16a** (*Z*)
Formula	$C_{13}H_{15}N_3O_4$	$C_{13}H_{15}N_3O_4$
Formula weight	277.28	277.28
Temperature (K)	293(2)	293(2)
Wave length (A)	1.5418	0.619
Crystal system	Monoclinic	Monoclinic
Space group	C2/c	P21/c
a (Å)	21.141(5)	6.225(5)
b (Å)	9.610(3)	17.885(5)
c (Å)	13.980(4)	12.157(5)
b (°)	107.33(8)	92.35(5)
V (A^3)	2711.3(13)	1352.4(13)
Z	8	4
Density, calcd (g cm^{-3})	1.359 g.cm^{-3}	1.362 g.cm^{-3}
Crystal habit	Needle (colourless)	Needle (colourless)
(m mm^{-1})	0.860	0.103
F(000)	1168	584
Crystal dimensions (mm)	0.18×0.08×0.06	0.14×0.02×0.01
q range for data collection (°)	10 to 24	17 < q < 14.1
Index range	$-24\leq h\leq 22$	$-3\leq h\leq 4$
	$0\leq k\leq 11$	$-9\leq k\leq 10$
	$0\leq 1\leq 16$	$-5\leq 1\leq 6$
Reflections collected	4443	739
Independant reflections (R_{int})	962(0.04)	343(0.10)
Refinement method	Full matrix 1-s on F^2	Constrained 1-s on F^2
Data/parameters	962/182	343/184
Goodness-of-fit	0.44	0.63
R-factor [$F^2 > 2s(F^2)$]	0.046 (603 reflections)	0.035 (343 reflections)
	0.056 (all reflections)	0.0492 (all reflections)
wR2 [1 < 2s(1)] (all intensities)		
x,y,w = $1/[s^2(F_o^2) + (xP)^2 + yP]$	x = 0.1706, y = 1.9204	x = 0.0625, y = 1.2693
where $p = (F_o^2) + 2F_c^2)/3$		
Extinction coefficient	None	None
Largest diff. peak and hole (e$Å^{-3}$)		0.109 and −0.091

11.7). Following removal of adhering fat and connective tissues, the tracheas were cut into four rings. The rings were suspended in 5 mL organ baths containing Krebs solution at 37°C, gassed with 95% O_2 and 5% CO_2 and equilibrated under an initial tension of 1.5 g. After equilibration for 1 h, the resting tension was between 0.6 and 1.4 g. Tension was measured isometrically with strain gauges (UF-1 Pioden) and amplifiers (EMKA, France) and displayed on recorders (Sefram, France).

Human isolated bronchus. Human bronchial tissues were removed from patients undergoing surgery for lung cancer. Just after resection, segments of bronchi with an inner diameter of 4–6 mm were taken from an area as far as possible from the malignancy. They were carefully dissected free of the parenchyma from distal lobar to segmental airways and transported to the laboratory in Krebs solution previously aerated with the gas mixture of 95% O_2 and 5% CO_2. The tissue was stored at 4°C until the experiment was carried out (2 to 24 h maximum), and two to eight rings of the same bronchus were prepared. Each bronchial ring was suspended in Krebs solution under an initial tension of 2 g and treated in every respect as the rat tracheal rings.

Protocols. Each experiment was initiated by inducing a maximal contraction of the tracheal or bronchial rings with acetylcholine (Ach 3 mM) and then a maximal relaxation with aminophylline 3 mM. This initial maximal contraction and relaxation was performed to stabilize the preparations. During the following hour, the tissues were washed every 15 min. Concentration–relaxation curves to oximino-caprolactames and references compounds (aminophylline, isoprenaline, cromakalim, salbutamol, verapamil) were obtained in preparations precontracted with Ach. After a stable level of precontraction, in response to a concentration of Ach giving 50% of the maximal response, the compounds were added to the bath in cumulative concentrations. Afterwards, aminophylline 3 mM was added to the bath to obtain the maximal relaxation. Only one concentration–response curve of each relaxant agent was obtained from each ring. Experiments were conducted on parallel groups of four to eight rings, with at least one ring serving as control.

The mechanism of oximino-caprolactame **(16b)**-induced relaxation was investigated on the rat trachea preparation by studying the effects of selective modulators of the different airway smooth muscle pathways of relaxation. The modulators were added to the Krebs solution 20 min before contraction with acetylcholine, i.e. at least 30 min before concentration response curve to **16b**. In order to test the potential action of **16b** as a calcium antagonist, we have also studied the inhibitory effect of this compound on the contraction induced by KCl, a depolarizing agent known to contract the airway smooth muscle by promoting the influx of extracellular

calcium, as well as on the contraction directly caused by extracellular calcium ($CaCl_2$) added to the bath in a depolarizing medium, according to Advenier et al.[13]

Expression of results and statistics. The relaxing effects of compounds are expressed as a percentage of the maximal relaxation produced by aminophylline (3 mM). IC_{50} was defined as the concentration of the agent that caused 50% of maximal relaxation to aminophylline 3 mM. For the studies on the inhibitory activity of **16b** on either KCl- or calcium-induced contraction, results are expressed as percentages of the maximal contraction induced either by KCl or by Ach (3 mM). Results are given as mean ± SEM. Statistical analysis was performed using the Student's t test. $p < 0.05$ values were considered as significant.

Drugs. The drugs used and their sources were: isoprenaline, aminophylline (PCH, Paris, France), salbutamol sulfate (Glaxo, Paris, France), acetylcholine, cromakalim, tetraethylammonium chloride (TEA), sodium nitroprusside (SNP), glibenclamide, methylene blue, dipyridamole, indomethacin, *N*-methylhydroxylamine, forskolin (Sigma, St. Louis, USA). The selective phosphodiesterase (PDE) inhibitors: siguazodan (PDE III inhibitor), rolipram (PDE IV inhibitor) and zaprinast (PDE V inhibitor) were generous gifts from Professor Advenier (Paris, France). All stock solutions of the chemicals were made in distilled water, excepting glibenclamide in methanol, indomethacin and rolipram in ethanol, siguazodan and forskolin in dimethyl sulfoxide (Sigma, St. Louis, USA) and zaprinast in ammonia (1.6% v/v). All the stock solutions were kept frozen until use and then diluted in distilled water. The oximino-caprolactame derivatives were all prepared in dimethyl sulfoxide (DMSO) (Sigma). At no time did the maximal concentrations of solvents exceed 0.3% by volume in the organ bath and produced by themselves any significant direct effect on the basal tone of the rat trachea or on responses to reference compounds.

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