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Synthesis of 1,2-benzisothiazole derivatives and investigation of their putative histaminergic activity

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Abstract

Some new 2-(1,2-benzisothiazol-3-yl)ethylamine derivatives were synthesised and their putative histaminergic activity was investigated in in vitro gastrointestinal and cardiac preparations. In the isolated guinea pig duodenum, all the compounds induced a tetrodotoxin- and atropine-sensitive contractile activity, which was minimally affected by mepyramine in the case of the compound 2-(1,2-benzisothiazol-3-yl)ethylamine. In the same tissue, all the compounds were devoid of any H_3 receptor agonistic or antagonistic activity, but caused a nicotinic and/or 5-HT_3 receptor activation. None of these compounds induced any histamine H_2 agonistic or antagonistic activity in the isolated guinea pig gastric mucosa or in the isolated papillary muscle. On this latter substrate, the compound *N*,*N*,*N*-trimethyl-2-(1,2-benzisothiazol-3-yl)ethylammonium iodide induced a positive inotropic activity, apparently due to a release of catecholamines. These results demonstrate the substantial inability of 1,2-benzisothiazole derivatives to interact with histamine receptors in functional tests. These compounds, however, possess gangliomimetic properties, related to the activation of $5HT_3$ and/or nicotinic receptors.

Keywords: 1,2-Benzisothiazole derivatives; Histamine receptors; Gastric mucosa; Duodenum; Papillary muscle; Gangliomimetic effect

1. Introduction

The class of benzisothiazoles includes compounds endowed with pharmacological activities in different animal tests. Molecules containing the 1,2-benzisothiazole nucleus possess anti-inflammatory and anaesthetic-like effects [1,2], while carboxyamide-substituted benzisothiazoles were found to induce spasmolytic activity in the isolated guinea pig ileum [3] and in the human myometrium [4], precontracted with acetylcholine and histamine, respectively. Moreover, some quaternary ammonium salts derived from benzisothiazole are endowed with analeptic respiratory activity and produce a rise in cardiovascular parameters, apparently through a nicotine-like gangliomimetic effect [5,6]. On the basis of these studies, it is evident that benzisothiazole derivatives can interact with different receptor systems in peripheral and central tissues.

Among the pharmacological properties of benzisothiazole derivatives, the anti-inflammatory effects [1] and the spasmolytic activity in histamine-contracted uterus [4] could be suggestive of interactions with histamine receptors, but, so far, the ability of these compounds to interfere with these receptors has not been fully explored. On the other hand, the benzisothiazole-based structures do not feature molecular characteristics of either agonists and antagonists of histamine H_1, H_2 or H_3 receptors [7]. In fact, the imidazole ring, or a closely related heterocycle, represents the prerequisite for the activity of histamine receptor ligands [7], although several important exceptions are known. Indeed, the histaminergic activity can reside in totally unexpected structures, like the recent histamine H_1 antagonists [7], or in the atypical neuroleptic drug clozapine and its congeners, which display a high affinity for histamine H_3 receptors [8].

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Recently, several thioperamide derivatives, where the cyclohexylcarbo–thioamide group was replaced by the benzothiazole nucleus (a structure related to benzisothiazole nucleus), were shown to bind to the histamine H_3 receptor site in the brain cortex [9]. Even though the affinity of these derivatives for the H_3 site was lower than that of thioperamide, their ability to produce histaminergic effects cannot be excluded.

On the basis of these considerations, we wanted to verify the ability of 1,2-benzisothiazoles to produce histamine receptor agonistic and/or antagonistic activity. To this aim, some new 2-(1,2-benzisothiazol-3-yl)ethylamine derivatives (compounds **4a**–**d**, see Scheme 1) were synthesised and biologically assayed in isolated preparations, suitable for the investigation of histamine receptor-mediated effects.

2. Chemistry

Benzisothiazole derivatives **4a**–**d** were prepared, as illustrated in Scheme 1, by means of nucleophilic substitution on 2-(1,2-benzisothiazol-3-yl)chloroethane (**3**) by a suitable amine. These reactions, performed in anhydrous EtOH, gave good yields.

2-(1,2-Benzisothiazol-3-yl)ethanol (**2**), prepared by reduction of 1,2-benzisothiazol-3-yl-acetic acid ethyl ester, was treated with $SOCl_2$ to obtain the chloroethane derivative **3**.

3. Chemical experimental

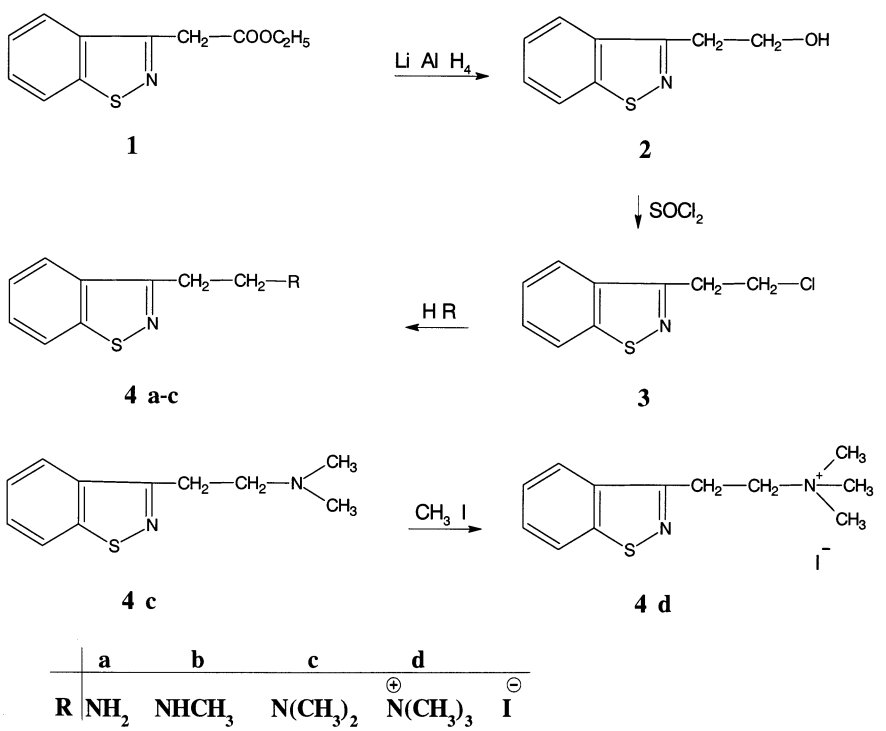
Melting points were determined on a Büchi–Tottoli apparatus and are uncorrected. The newly synthesised substances were analysed for C, H and N. The percentages found were within $\pm 0.4\%$ of theoretical values. Characteristic data and yields of intermediates and final products are reported in Table 1.

The ^{1}H NMR (samples were analysed in CD_3SOCD_3 or otherwise as indicated) and mass spectra data were recorded on Bruker 300 (300 MHz) and Finnigan 1020 instruments, respectively. The spectral data of synthesised compounds are reported in Table 2.

Reactions were followed by TLC, on a kieselgel 60 F 254 (DC-Alufolien, E. Merck).

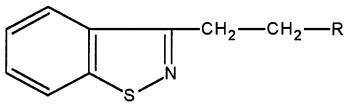
*3.1. 2-(1,2-Benzisothiazol-3-yl)ethanol (**2**)*

A mixture of 0.21 g (0.0055 mol) of $LiAlH_4$ in 20 ml of anhydrous Et_2O was stirred and added slowly to an ether solution of 1.1 g (0.005 mol) of 1,2-benzisothiazol-3-yl-acetic acid ethyl ester, prepared according to the method described by Vitali et al. [10]. The mixture was stirred at room temperature for 10 h, 2N H_2SO_4 was added and the mixture was extracted with Et_2O. The solvent was removed under reduced pressure and the residue was purified by distillation under vacuum. Yield 78%; b.p. 116–120°C/0.35 mm.



Scheme 1. Synthetic pathways.

Table 1
Characteristic data of intermediates and final products



Comp.	R	Crystallisation solvent	B.p. (°C)/mm or m.p.	Yield (%)	Analysis
2	OH		116–120/0.35	78	C_9H_9NOS
3	Cl	petroleum ether	65–66	74	C_9H_8ClNS
4a · HCl	NH_2	abs. EtOH	174–176	78	$C_9H_{11}ClN_2S$
4b · HCl	$NHCH_3$	abs. EtOH	223–224	75	$C_{10}H_{13}ClN_2S$
4c · HCl	$N(CH_3)_2$	abs. EtOH	207–208	72	$C_{11}H_{15}ClN_2S$
4d	$N^+(CH_3)_3I^-$	abs. EtOH	246–248	88	$C_{12}H_{17}IN_2S$

*3.2. 2-(1,2-Benzisothiazol-3-yl)chloroethane (**3**)*

To 10 ml of $SOCl_2$ was added 1.79 g (0.01 mol) of 2-(1,2-benzisothiazol-3-yl)ethanol (**2**) and the mixture was refluxed for 1 h. The excess $SOCl_2$ was evaporated under reduced pressure and the residue was crystallised from petroleum ether. Yield 74%; m.p. 65–66°C.

*3.3. General procedure for the preparation of 2-(1,2-benzisothiazol-3-yl)ethylamine derivatives (**4a–c**)*

Compounds **4a–c** were prepared according to Scheme 1. In an autoclave an anhydrous EtOH solution of the appropriate amine was added to 1.97 g (0.01 mol) of 2-(1,2-benzisothiazol-3-yl)chloroethane (**3**) and heated at 80°C for 12 h. The mixture was then decoloured, filtered and evaporated under reduced pressure. The final products were characterised as hydrochlorides. The yields and characteristics of compounds **4a–c** are reported in Table 1.

*3.4. N,N,N-Trimethyl-2-(1,2-benzisothiazol-3-yl)ethylammonium iodide (**4d**)*

A solution of 1.21 g (0.005 mol) of *N*,*N*-dimethyl-2-(1,2-benzisothiazol-3-yl)ethylamine (**4c**) in 40 ml of anhydrous EtOH was added to 0.85 g (0.006 mol) of CH_3I and allowed to stand at room temperature for a night. At the end of this time the quaternary salt was separated by filtration and crystallised from anhydrous EtOH. Yield 88%; m.p. 246–48°C.

4. Pharmacological experimental

Several biological substrates in vitro, taken from guinea pig, were prepared and used for testing compounds **4a–d**.

(a) Duodenum. Duodenal segments of whole duodenum (25–30 mm) were excised from male guinea pigs and bathed in organ chambers containing a modified Krebs–Heinseleit solution, according to a previously described method [11]. Preparations were connected to an isotonic transducer for the measurement of longitudinal muscle contractions. This kind of preparation can be used as a test to evaluate histamine H_1 and H_2 receptor-evoked contractions [11]. In some experiments, duodenal preparations were electrically driven by means of a pair of coaxial platinum electrodes, connected to an electronic stimulator, delivering square-wave pulses (0.1 Hz, 0.5 ms, 200–250 mA). Electrical field stimulation (EFS) was proven to evoke cholinergic nerve-mediated contractions, which can be negatively modulated by presynaptic histamine H_3 receptors [11].

Table 2
Spectral data: NMR spectra of newly synthesised compounds and mass spectra (principal peaks) of final products

Comp.	
2	NMR (CCl_4): δ 7.88–7.68 (m, 2H, Ar), 7.44–7.18 (dt, 2H, Ar), 3.96 (t, 2H, CH_2), 3.37 (sb, 1H, OH), 3.16 (t, 2H, CH_2)
3	NMR (CCl_4): δ 7.84–7.66 (m, 2H, Ar), 7.44–7.18 (dt, 2H, Ar), 3.93 (t, 2H, CH_2), 3.42 (t, 2H, CH_2)
4a	NMR (CD_3SOCD_3): δ 8.23–8.15 (dd, 2H, Ar), 8.04 (b, 2H, NH_2), 7.67–7.52 (dt, 2H, Ar), 3.46 (t, 2H, CH_2), 3.34 (t, 2H, CH_2). Mass: 178 (7, M^+), 149 (100)
4b	NMR (CD_3SOCD_3): δ 9.03 (sb, 1H, NH), 8.23–8.18 (dd, 2H, Ar), 7.67–7.52 (dt, 2H, Ar), 3.52 (t, 2H, CH_2), 3.43 (t, 2H, CH_2), 2.62 (s, 3H, CH_3). Mass: 192 (13, M^+), 149 (100)
4c	NMR (CD_3SOCD_3): δ 8.25–8.21 (dd, 2H, Ar), 7.67–7.53 (dt, 2H, Ar), 3.61 (s, 4H, CH_2), 2.86 (s, 6H, CH_3). Mass: 206 (5, M^+), 58 (100)
4d	NMR (CD_3SOCD_3): δ 8.27–8.22 (m, 2H, Ar), 7.69–7.56 (dt, 2H, Ar), 3.95 (t, 2H, CH_2), 3.67 (t, 2H, CH_2), 3.21 (s, 9H, CH_3). Mass: 206 (7, M^+), 58 (100)

Therefore, the guinea pig duodenum represents a good pharmacological substrate for testing ligands acting at these receptors, as well as at other presynaptic receptors.

(b) Papillary muscle. Left papillary muscles were excised, set up into isolated organ chambers containing a modified Ringer solution and electrically paced (2 Hz, 10–30 mA) by means of two platinum electrodes implanted into the ventricular basis. As previously described [13], this kind of preparation represents an elective test for studying histamine H_2 receptors. In fact, histamine and H_2 receptor agonists evoke positive inotropic activity, which can be antagonised by specific antagonists [11].

(c) Gastric mucosa. A classical procedure was followed [13]. The gastric mucosa was separated from the muscular layer of the guinea pig stomach and the secretory portion was mounted on the end of a plastic funnel (1 cm^2). The preparation was then immersed from the serosal side into a buffered nutrient solution (pH 7.2), in which the drugs tested were administered. A non-buffered solution was added to the mucosal side, allowing pH determination and titration. It is known that the activation of histamine H_2 receptors in the stomach is associated with a rise in acid secretion [13]. The ability of drugs to evoke secretory effects was then measured in terms of μEq of HCl produced by the mucosal specimen in 10 min.

5. Results and discussion

(a) Guinea pig duodenum. Compounds **4a**–**c** did not modify the basal tone of the preparations up to 0.1 mM. These compounds, at higher concentrations (1 mM) and compound **4d** (0.01–1 mM) caused a contractile effect, which was totally insensitive to histamine H_2 receptor blockade by famotidine (10 μM) and, in the case of compounds **4a** and **4b**, slightly affected by the H_1 receptor blocker mepyramine (0.1 μM) (Fig. 1). It is evident that all these compounds do not induce any H_2 receptor-mediated activation of the intestinal smooth muscle, while compounds **4a** and **4b** may cause an erratic activation of excitatory histamine H_1 receptors. The contractile effect of compounds **4a**–**d** was prevented by atropine (0.1 μM) or by tetrodotoxin (1 μM), suggesting that they act through a nerve-mediated effect and that the final mediator producing muscle contrac-

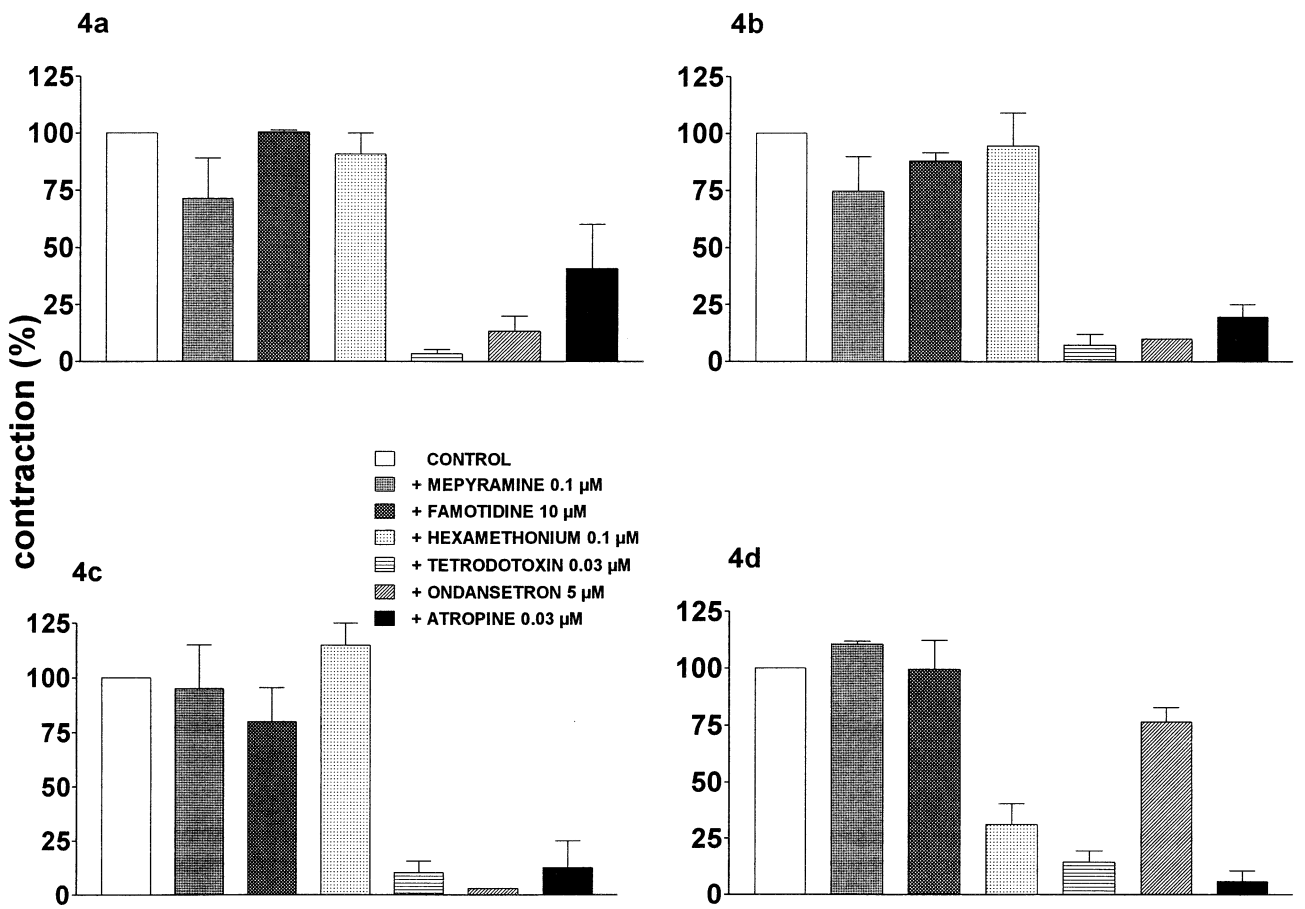


Fig. 1. Guinea pig duodenum. Effect of different antagonists on muscle contractions elicited by compounds **4a**–**d**. Data are the mean ± SEM of four to six experiments.

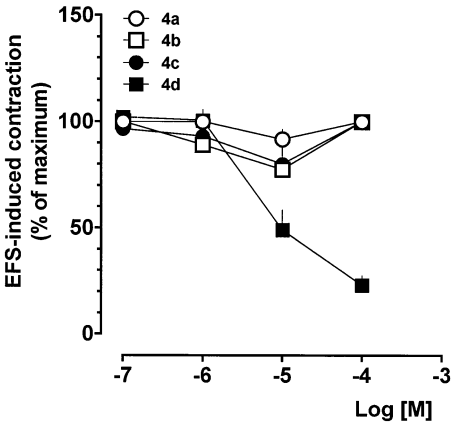


Fig. 2. Guinea pig duodenum. Inhibitory activity of compounds **4a**–**d** on muscle contractions elicited by EFS. Data are the mean ± SEM of five experiments.

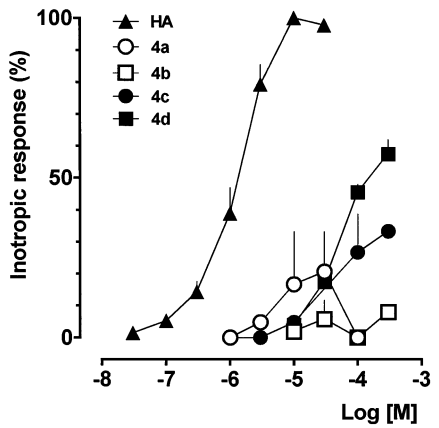


Fig. 3. Papillary muscle. Positive inotropic effect of histamine (HA) and of compounds **4a**–**d**. Results are expressed as percentage of the maximum effect of histamine. Data are the mean ± SEM of five to six experiments.

tion is represented by acetylcholine (Fig. 1). Furthermore, the contractile effect of compound **4d** was prevented by hexamethonium (0.1 mM), suggesting that such a benzisothiazole derivative, perhaps by virtue of the quaternary ammonium group on the alkyl chain, possesses nicotine-like gangliomimetic properties. Conversely, the contractile effect of compounds **4a**–**c** proved to be sensitive to ondansetron, a selective antagonist of $5HT_3$ receptors [14].

When tested on preparations precontracted with acetylcholine, compounds **4b**–**d** revealed a certain degree of relaxant activity at concentrations higher than 10 μM, while compound **4a** was virtually inactive (data not shown). Conversely, only compound **4d** (3–100 μM) inhibited EFS-induced contraction (Fig. 2), but its effect was not prevented by the histamine H_3 receptor antagonist thioperamide [11] (data not shown). Moreover, none of the compounds investigated was able to modify the inhibitory activity of $R(\alpha)$-methylhistamine, a specific H_3 receptor agonist [11]. These findings exclude an effect of compounds **4a**–**d** at histamine H_3 receptors. The inhibitory activity of compound **4d** on EFS-evoked contraction represents a direct effect on the smooth muscle, as already observed with other benzisothiazoles [3,4].

(b) Papillary muscle. Compounds **4a** and **4b** were virtually devoid of inotropic activity up to 0.3 mM, giving 15 and 5% of the maximum positive inotropic response to histamine (Fig. 3). Conversely, compounds **4c** and **4d** showed a higher degree of positive inotropic activity (Fig. 3), giving 37 and 62% of the maximum effect of histamine, respectively. However, the positive inotropic effect of compounds **4c** and **4d** was not modified by the H_2 receptor antagonist famotidine, even at concentrations (10 μM) known to cause a 100-fold rightward shift of the histamine concentration response curve [12]. None of these compounds, in turn, modified the response to histamine, even when 1 mM concentrations were applied (data not shown). It is evident that these compounds are devoid of both ago-

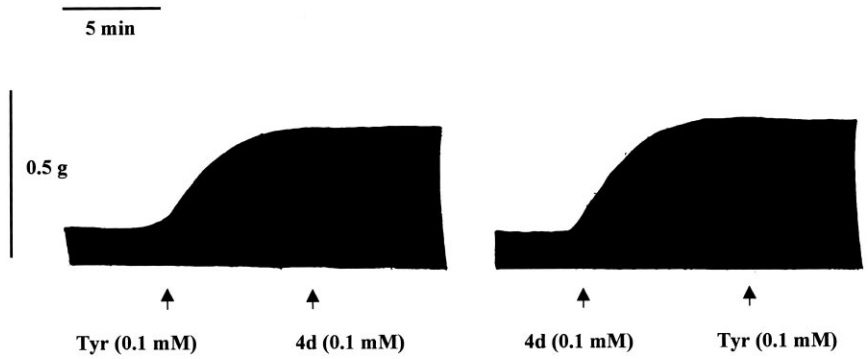


Fig. 4. Papillary muscle. Original recording showing the positive inotropic effect of tyramine and compound **4d**, measured in the same preparation. The figure is representative of four experiments.

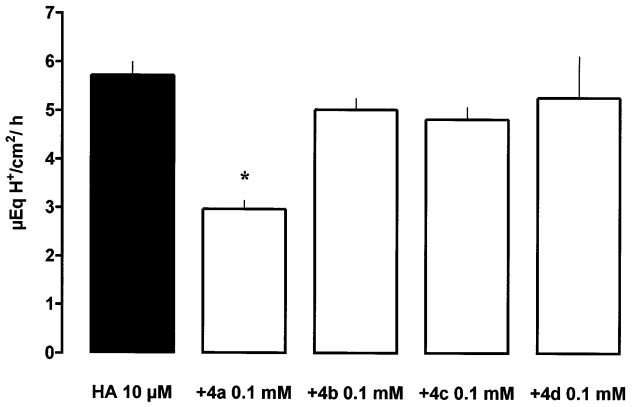


Fig. 5. Gastric mucosa. Effects of compounds **4a**–**d** on histamine (HA)-induced hypersecretion. Black bar is histamine alone; white bars represent histamine in the presence of compounds **4a**–**d**. Data are the mean ± SEM of four to five experiments.

nistic and antagonistic activity at histamine H_2 receptors, thus confirming the results in duodenum.

The positive inotropic effect of the more active compound **4d** was better investigated, in an attempt to understand the intimate mechanism of action. This effect of the compound was virtually abolished by the competitive β-blocking drug propranolol [14] (100 nM), but the antagonism was apparently of the insurmountable type, not being reversed by a rise in the concentration of compound **4d** up to 1 mM (data not shown). The effect of such a compound was also abolished in the presence of maximum concentrations of the catecholamine releaser tyramine (0.1 mM). Similarly, the positive inotropic activity of tyramine was abolished by maximum concentrations of compound **4d** (Fig. 4). These data suggest that this compound acts through an indirect effect, mediated by catecholamines released from tissue stores. The mechanism could be similar to that of tyramine, whose effect is usually inhibited by competitive β-blocking drugs in an insurmountable way [15].

(c) Gastric mucosa. Differently from histamine and for other H_2 receptor agonists [13], compounds **4a**–**d** did not induce any acid secretory effect when tested in the range of concentrations 1–100 mM (data not shown). Compound **4a** (0.1 mM), but not the others, caused a partial damping of the histamine-induced secretory activity (Fig. 5). The inability of this compound to induce any antagonistic activity at histamine H_2 receptors in the heart and duodenum (see above) makes it unlikely that histamine H_2 receptors have any involvement.

6. Conclusions

Taken together, these results demonstrate that the 1,2-benzisothiazole-based compounds possess a negligible activity at histamine receptors. However, compounds **4a**–**c** manifest an interesting gangliomimetic activity, which may depend on 5-HT_3 or nicotinic receptor stimulation. The correlation of these structures with the serotonin molecule could justify the observed biological effects. Recently, it has been shown that some benzisothiazole derivatives can also act at $5HT_{1a}$ and $5HT_2$ receptors [16,17], even though it is not clear which molecular determinant in these compounds is crucial for such interaction.

Due to the importance of 5-HT_3 receptors in the control of intestinal motility, a better characterisation of the molecular determinants that favour such interaction is desirable. Consequently, the synthesis of more potent and specific benzisothiazole derivatives endowed with serotoninergic activity could constitute a new family of drugs to be used as a tool in experimental studies of gastrointestinal motility.

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