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SAR studies on H_2 antagonists containing alkylamino substituted 1,2,5-thiadiazole 1-oxide moieties

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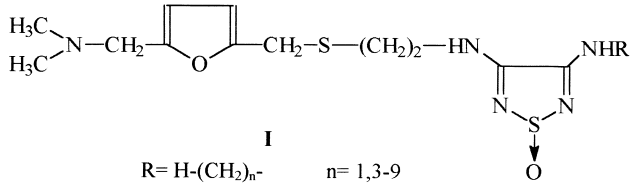
Abstract

A number of ranitidine analogues in which the diamino-1,2,5-thiadiazole 1-oxide substructure bearing alkyl chains of different length is present as the urea equivalent group, were synthesised and studied for their lipophilic and H_2 antagonist properties. Derivatives which displayed a log$P \leq 3$ behaved as competitive antagonists of histamine at H_2 receptors present on guinea pig right atrium. The remaining more lipophilic members of the series showed an insurmountable antagonism not completely reversible after prolonged washing. A binding study suggested that an increase in the length of alkyl chain gave rise to hydrophobic interactions with the receptor which were responsible for the apparent irreversible H_2 antagonism shown by the higher homologues of the series.

Keywords: H_2 antagonists; Ranitidine analogues; SAR in H_2 antagonists

1. Introduction

In the past few years many H_2 antagonists have been synthesised and screened for their structure–activity relationships. These studies showed that the structure of a classical H_2 antagonist is formed by an aromatic ring linked through a flexible chain to a planar π-electron moiety called the 'urea equivalent' group. This last substructure has to be very polar and to display both α and β solvatochromic properties [1]. More recent results suggest the presence at the H_2 receptor of a hydrophobic binding area adjacent to the site fitted by the 'urea equivalent group' [2–5]. In order to have more information on the potential role of this site in the design of new H_2 antagonists a series of ranitidine analogues with general formula **I** were synthesised and studied for their lipophilicity and pharmacological properties.



I

R= H-$(CH_2)_n$- n= 1,3-9

These compounds have as 'urea equivalent' group the diamino-1,2,5-thiadiazole 1-oxide substructure, substituted on the lateral amino function with alkyl chains of increasing length. This implies a deep modulation of the lipophilicity in the molecular area which should interact with the additional binding site.

2. Chemistry

The common intermediate **3** used for the preparation of the final derivatives (Scheme 1) was synthesised by action of 2-(5-(dimethylaminomethyl)furfurylthio)ethaneamine (**1**) on 3,4-dimethoxy-1,2,5-thiadiazole 1-oxide (**2**) in methanol solution. The crude product so obtained, treated with an excess of the appropriate amine in methanol solution, afforded the final compounds **4a–h**, which were characterised by standard methods.

Partition coefficients in *n*-octanol/water of derivatives **4a–h** were calculated using the CLOGP algorithm [6] starting from the experimental value of **4a**. The validity of this approach was checked by measuring log *P* of selected members of the series by potentiometric titration method, using the Sirius PCA 101 instrument. We found good agreement between the measured and calculated values (Table 1).

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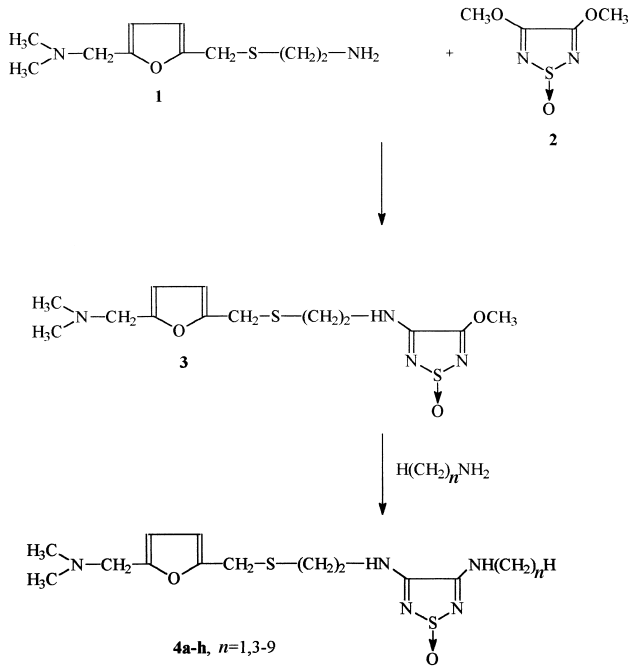

Scheme 1. Synthesis of compounds **4a–h**.

3. Chemical experimental

Melting points were determined on a Büchi 530 capillary melting point after introducing the sample into the bath at a temperature 10°C lower than the melting point, heating rate 1°C min^{-1}, 3 °C min^{-1} in the case of decomposition. All of the compounds were routinely checked by IR (Shimadzu FTIR-9101 M), mass spectrometry (Finningan-Mat TSQ-700), ^{1}H and ^{13}C NMR (Bruker AC-200). Derivatives **1** [7], **2, 3, 4a** [8] were prepared according to the methods reported in the literature. Microanalyses were performed by Redox Cologno M. (MI): carbon, hydrogen and nitrogen results were within ± 0.4% of theoretical values. Partition coefficients were calculated using the Windows program (BioByte Corp.), version 1.0.0. Partition coefficients of derivatives **4e–g** were measured by a two-phase titration method (Sirius) [9].

*3.1. General method of preparation of 3-alkylamino-4-(2-((5-dimethylaminomethyl-2-furyl)methylthio)ethylamino-1,2,5-thiadiazole 1-oxide derivatives (**4a–h**)*

To a stirred solution of **2** (1.0 g, 6.17 mmol) dissolved in methanol (100 ml), a solution of **1** (1.32 g, 6.17 mmol) in methanol (10 ml) was added dropwise. The mixture was kept under stirring for 30 min at room temperature, concentrated to 30 ml, and then 12.3 mmol of the appropriate alkylamine were added dropwise under stirring. After 30 min in vacuo solvent removal gave an oil which was purified on a silica gel column (Merck, Kieselgel 60), 70–230 mesh ASTM; eluent, dichloromethane/methanol 2%. The oils obtained after chromatographic purification were immediately transformed into the corresponding oxalates, which were crystallised from methanol/isopropyl alcohol. Melting points and yields are reported in Table 1.

4. Pharmacology

All the compounds were examined for antagonism of the positive chronotropic action of histamine on isolated guinea pig atria, using the procedure described by Black et al. [10]. Selected compounds were also tested on gastric fundus isolated from immature rats according to the procedure previously described [11].

Specificity for H_2 receptors was evaluated on the basis of the ability of the compounds to antagonise the ileum contraction induced by histamine (H_1 receptors) and carbamoylcholine (muscarinic receptors), respectively. Binding

Table 1
Physical and pharmacological data of compounds **4a–h**

Compound	Yield (%)[a]	M.p. (°C)[a]	CLOGP	Right atrium	
				pA_2 ± 95% CL	Slope of Schild plot ± 95% CL
4a	76	132–134[b]	(0.89)[c]	7.03 ± 0.15	1.05 ± 0.22
4b	75	156–158[b]	1.948	7.14 ± 0.23	1.03 ± 0.23
4c	77	151–153[b]	2.477	7.37 ± 0.22	1.03 ± 0.23
4d	73	140–141[b]	3.006	7.65 ± 0.12	1.04 ± 0.16
4e	71	90–91	3.535 (3.46)[c]	8.15[d]	NC[e]
4f	60	100–101	4.064 (3.91)[c]	7.89[d]	NC
4g	62	105–107	4.593 (4.47)[c]	7.30[d]	NC
4h	68	102–104	5.122	7.09[d]	NC

[a] As oxalate.
[b] Decomposition.
[c] Experimental value.
[d] pA_2 value calculated from the equation $-\log K_B = -\log(B/DR - 1)$ [14] for antagonists **4e–h** at concentration 10^{-7} M, which causes only a non-significant reduction of the maximum in the concentration–response curves of histamine.
[e] NC, not calculated.

studies on derivative **4e** were performed with homogenised guinea pig cortex membranes, using the procedure described by Gajtkowski et al. [12].

5. Pharmacological experimental

5.1. Guinea pig right atria preparation

The hearts were rapidly removed and the atria were suspended in organ baths containing 30 ml of Ringer–Locke modified solution of the following composition (mM): NaCl 154, KCl 5.9, $CaCl_2$ 1.5, $NaHPO_4$ 0.29, $NaHCO_3$ 4.2, glucose 8.3, maintained at 31°C and gassed with O_2.

Tissues were allowed to equilibrate for 2 h and then a cumulative concentration–response curve to histamine was constructed recording right atria beating frequency after each agonist addition (subsequent additions were made only after the response to the previous histamine concentration had attained a maximal level and a steady beating frequency had been recorded). Following 1 h washing, tissues were incubated with the antagonist for 30 min and a second concentration–response curve to the agonist was obtained. Tissues were then washed every 20 min for 1 h or 3 h and a third concentration–response curve to the agonist was performed.

5.2. Isolated gastric fundus from immature rats

Weaned male immature rats (Wistar, 30–35 g) were used. The gastric fundus was set up at 34°C in a Perspex tube with the mucosa facing into the lumen, bathed with 5 ml of oxygenated (100% O_2) unbuffered solution, whereas the serosal surface was bathed with 30 ml buffered solution gassed with 95% O_2 and 5% CO_2. The composition of the serosal solution was (mM): NaCl 110, $NaHCO_3$ 26, KCl 5, $CaCl_2$ 2.4 and glucose 16.7; the mucosal solution contained (mM): NaCl 136, KCl 5, $CaCl_2$ 2.4, $MgCl_2$ 2.4 and glucose 16.7. The acid output was measured by titrating mucosal samples (taken at 15 min intervals) to pH 7 with 10 mM NaOH. After an equilibration time of about 120 min, a submaximal concentration of histamine (4×10^{-5} M) was added to the serosal solution and left in contact with the stomach for 60 min. After washing, a second administration of histamine 4×10^{-5} M was given in control stomachs, whereas in treated stomachs, the antagonist was administered 60 min before the stimulant. Acid responses were reported as the percentage of control histamine response considered arbitrarily to be 100.

5.3. Binding of [^{3}H]tiotidine to guinea pig cerebral cortex

Guinea pig cerebral cortex membranes were removed and homogenised in 50 mM sodium–potassium phosphate buffer (pH 7.4), using an Ultra-Turrax homogeniser and centrifuged at 50 000 g for 10 min. The resulting pellet was washed three times by resuspension in phosphate buffer followed by recentrifugation. The final pellet was resuspended in 15 ml phosphate buffer (pH 7.4).

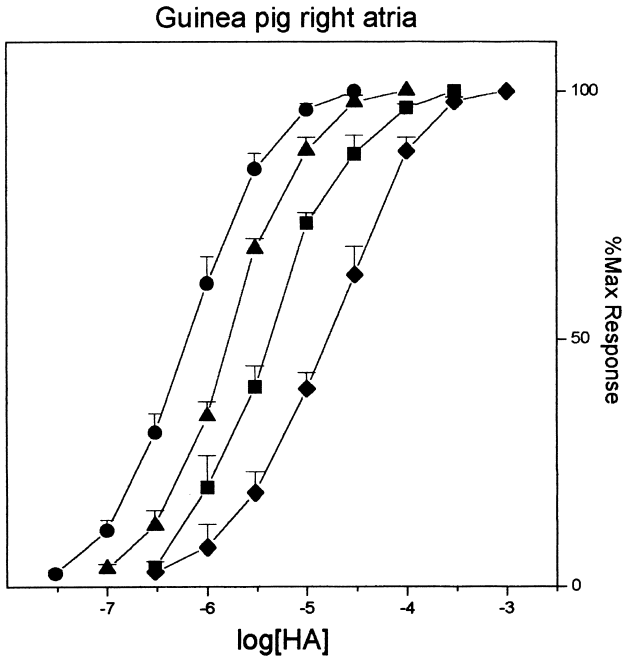

Fig. 1. Concentration–response curves of histamine on guinea pig right atria evaluated in the absence (●) and in the presence of compound **4c** at concentration 1×10^{-7} M (▲), 3×10^{-7} M (■) and 1×10^{-6} M (◆). Mean values ± SEM from 4–6 observations.

5.3.1. Irreversibility experiments

Three aliquots of this suspension (4.5 ml each) were incubated for 40 min at 30°C with the addition of either only buffer (control) or a solution 0.1 or 1 μM of compound **4e** to a final volume of 5 ml.

The membranes were then diluted to approximately 30 ml with ice-cold buffer, incubated a further 10 min on ice, centrifuged at 50 000 g for 10 min, and washed again by a series of four resuspensions and centrifugations [13].

5.3.2. Saturation receptor assay

Guinea pig cortical membranes (final concentration 2 mg ml^{-1}) were incubated for 1 h in phosphate buffer (50 mM, pH 7.4, $T = 30$°C) with seven different concentrations of [^{3}H]tiotidine (~ 87 Ci $mmol^{-1}$, NEN DuPont) in the range 0.78–50 nM, in the absence (total binding) and in the presence (non-specific binding) of 1×10^{-4} M cimetidine. Specific binding was calculated as the difference between total and non-specific binding. The reaction was stopped by adding 3 ml of ice-cold phosphate buffer to the assay volume (250 μl) and the mixture was filtered under reduced pressure onto Whatman GF/B glass-fibre filters, followed by three additional washes with ice-cold buffer. The amount of radioactivity retained on the filters was quantitated by liquid scintillation counting, using a Beckman liquid scintillation spectrophotometer.

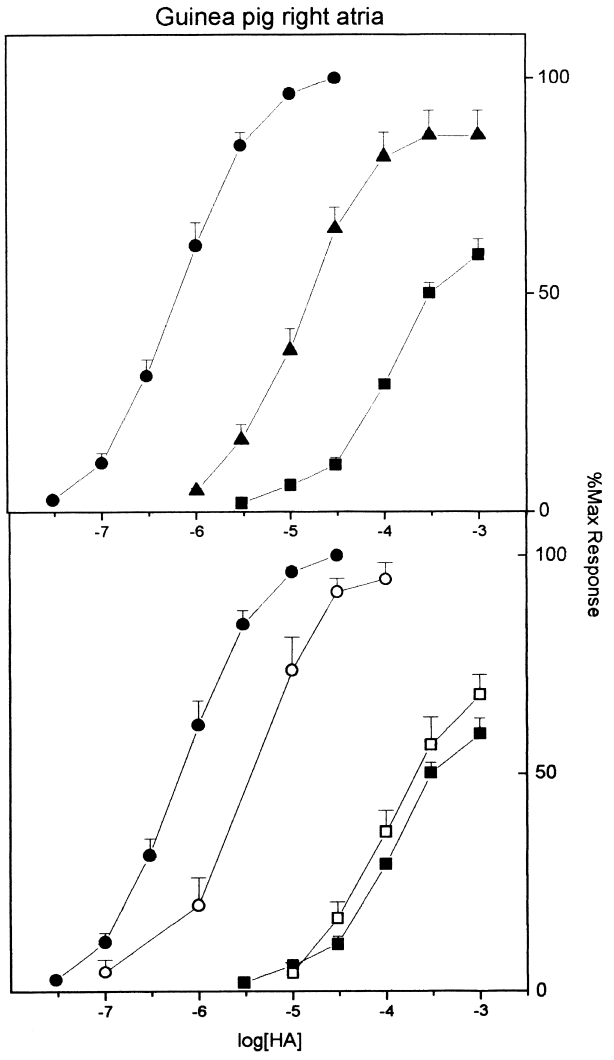

Fig. 2. (Top) Concentration–response curves of histamine on guinea pig right atria evaluated in the absence (●) and in the presence of compound **4e** at concentration 1×10^{-7} M (▲) and 1×10^{-6} M (■); (bottom) response recovery to histamine of guinea pig right atria preincubated with **4e** 1×10^{-6} M (■) after 1 h (□) and 3 h (○) of washing. Mean values ± SEM from 4–6 observations.

6. Biological results and discussion

In the experiments on the right guinea pig atria, derivatives **4a–d** ($n = 1, 3$–5) behaved as competitive antagonists of histamine at H_2 receptors. In fact they were able to shift the concentration–response curves for histamine without reduction of the maximal response, when tested in the concentration range 10^{-7} to 10^{-6} M (**4a–c**) or 3×10^{-8} to 3×10^{-7} M (**4d**) (Fig. 1).

The pA_2 values obtained by Schild analysis of the data (Table 1) show that, on increasing of the length of the lateral alkyl chain and consequently on increasing of the molecular lipophilicity, these compounds display a small increase in their affinity for H_2 receptors.

This trend is evident only when the two farthest members (**4a,d**) of the set are compared. Derivatives **4e–h** ($n = 6$–9), which display a log$P > 3.5$ behaved differently. In fact the cumulative concentration–response curves of histamine, in the presence of these compounds, were again shifted to the right but, starting from 10^{-7} M concentration, a progressive decrease of the maximal effect, not completely reversible after prolonged washing (1–3 h), was observed. This behaviour is depicted in Fig. 2 in the case of derivative **4e** ($n = 6$).

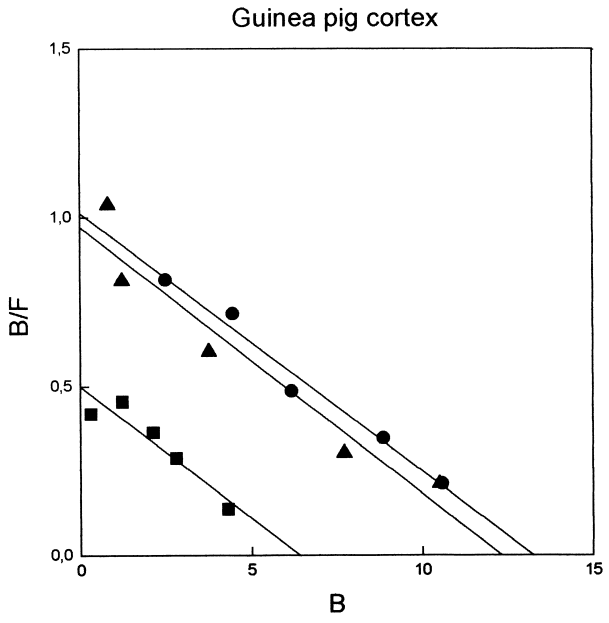

Fig. 3. Scatchard plot of specific [^{3}H]tiotidine binding of membrane preparation from guinea pig cortex pretreated with no drug (●; $K_d = 14.3 \pm 0.9$ nM, $B_{max} = 13.5 \pm 1.7$ fmol/mg protein) and with compound **4e** at concentration 1×10^{-7} M (▲; $K_d = 12.9 \pm 0.6$ nM, $B_{max} = 12.7 \pm 1.6$ fmol/mg protein) and 1×10^{-6} M (■; $K_d = 13.6 \pm 0.8$ nM, $B_{max} = 5.6 \pm 0.5$ fmol/mg protein).

The apparent affinity for H_2 receptors of this set of compounds (apparent pA_2, Table 1) decreased on increasing of the alkyl chain length. In order to better understand the apparent irreversible antagonism shown by a few members of the series, we subjected derivative **4e** to a binding study. Preincubation of rat cerebral cortex membranes with this compound (10^{-6} M) followed by a series of four subsequent suspensions and centrifugations of cortical membranes, and then assaying for saturation of [^{3}H]tiotidine binding, established that it is able to bind, in an apparently irreversible manner, to the H_2 receptors present on these membranes. Scatchard plots of saturation data showed about 58% of decrease in the number of H_2 receptors, without change in the affinity of the remaining sites for tiotidine (Fig. 3).

These results suggest that, in this series of compounds, the increasing of the length of the lateral aliphatic chain gives rise to hydrophobic interactions, which are very difficult to destroy by washing, with the additional binding area. This binding area is adjacent to the site fitted by the diamino-1,2,5-thiadiazole 1-oxide substructure, the common 'urea equivalent' group of the series. The small increase in affinity displayed by the lower terms of the series

and then the decrease shown by the higher ones, could indicate in these compounds the influence of a transport process or of hydrophobic interactions with the receptor which, as a whole, imply the existence of an optimum in the hydrophilic-lipophilic balance. Also the selectivity of compounds **4a–h** is dependent on the lipophilicity of each member. The first terms of the series **4a–d** at concentration 10^{-5} M were unable to inhibit the contractile responses mediated by histamine and carbamoylcholine, respectively, on the guinea pig ileum. By way of contrast, derivatives with $n > 4$ showed an insurmountable antagonism towards the two agonists.

In the light of these results we re-examined some of our previous data on H_2 antagonist properties (evaluated on guinea pig right atrium) of ranitidine analogues bearing the diaminofurazan substructure, substituted with alkyl moieties, as the 'urea equivalent' group [2–5]. In effect the more lipophilic members, namely those in which either cyclohexyl or phenylpropyl moiety was present at the lateral amino group, showed an insurmountable antagonism, when tested in the concentration range 10^{-7}–10^{-6} M. Therefore the quoted pA_2s for these compounds have to be considered only empirical values and not equal to $-\log K_B$.

Selected compounds, **4b**,**d–h**, were tested also on gastric fundus isolated from immature rats to evaluate their ability to inhibit acid secretion induced by histamine 4×10^{-5} M. When administered at a concentration of 1 or 10 μM 60 min before histamine, derivatives **4b**,**d–g** significantly reduced the acid response, whereas derivative **4h** (10 μM) was ineffective (Fig. 4).

Therefore, in this test, the increase of the lateral chain is accompanied by a progressive reduction of antagonism towards histamine until a complete disappearance occurs, and this again might be related to the increased lipophilicity

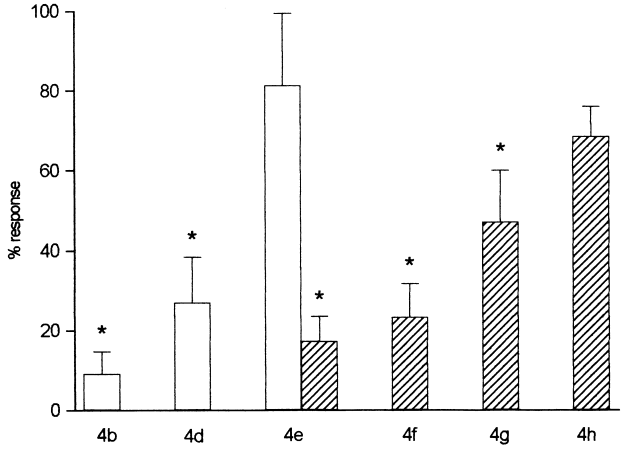

Fig. 4. Isolated gastric fundus from immature rats. Antagonism of the secretory response to histamine by a series of H_2 antagonists. Columns refer to secretory responses to histamine (4×10^{-5} M) in the presence of the H_2 antagonists administered at 1×10^{-6} M (open bars) or 1×10^{-5} M (diagonal bars). Control response to histamine in the absence of antagonists was considered arbitrarily as 100. Values are mean ± SEM from 6–8 observations. $^*P < 0.05$ versus controls.

of these compounds; the highly lipophilic H_2 antagonist zolantidine, in fact, was found to be completely inactive in rat gastric fundus, despite a noticeable antagonistic activity in isolated heart preparations [15].

In conclusion, these results show that this class of derivatives behave as weak inhibitors of HCl secretion induced by histamine in immature rat gastric fundus and that increasing the length of the alkyl chain linked to the diamino-1,2,5-thiadiazole 1-oxide over $n = 5$ affords compounds which act as apparently irreversible antagonists on H_2 receptors of guinea pig right atrium.

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