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Short communication

A potent gastric acid anti-secretory histamine H_2 receptor antagonist, *N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]-ethyl]thieno[3,4-*d*]isothiazol-3-amine 1,1-dioxide, hydrochloride (*W*y-45,727)

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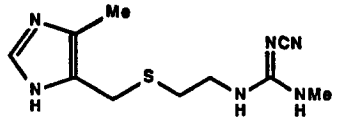
Summary — The synthesis of the title compound, a new histamine H_2-receptor antagonist ($pA_2 = 8.2$), (Wy-45,727; **7**) is described. Its gastric acid anti-secretory potencies in the rat and dog were found to be significantly greater than those of cimetidine or ranitidine.

Résumé — **Un puissant anti-sécrétoire de l'acide gastrique, antagoniste du récepteur H_2 de l'histamine: le chlorhydrate de *N*-[[[[(diméthylaminométhyl)-5 furanyl-2]méthyl]thio]éthyl]-2 thiéno[3,4-*d*]isothiazolamine-3 dioxyde 1,1.** *La synthèse de ce composé, un nouvel antagoniste du récepteur H_2 ($pA_2 = 8.2$), (Wy-45,727; 7) est décrite. Il a été démontré que sa puissance anti-sécrétrice d'acide gastrique chez le Rat et le Chien est significativement plus grande que celle de la cimétidine ou de la ranitidine.*

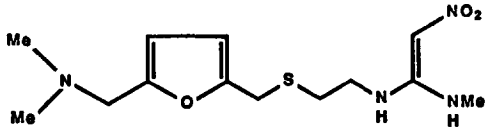
anti-secretory / H_2-receptor antagonist / *N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]thieno[3,4-*d*]-isothiazol-3-amine 1,1-dioxide hydrochloride / Wy-45,727

Introduction

Since the introduction of cimetidine (Tagamet®) [1], an H_2-receptor antagonist used in the treatment of gastric and duodenal ulcers, there has been intense activity in this area of therapy in efforts to design drugs with improved potency and efficacy. Ranitidine (Zantac®), the second drug in this class available clinically, showed a marked improvement in potency over cimetidine as a gastric acid anti-secretory agent [1]. We now report the synthesis and gastric anti-secretory activity of a still more potent member of this H_2-receptor class of drugs, *N*-[2-[[[5-[(dimethyl-amino)methyl]-2-furanyl]methyl]thio]ethyl]thieno[3,4-*d*]iso - thiazol-3-amine 1,1-dioxide, hydrochloride (Wy-45,727; **7**) [2, 3].



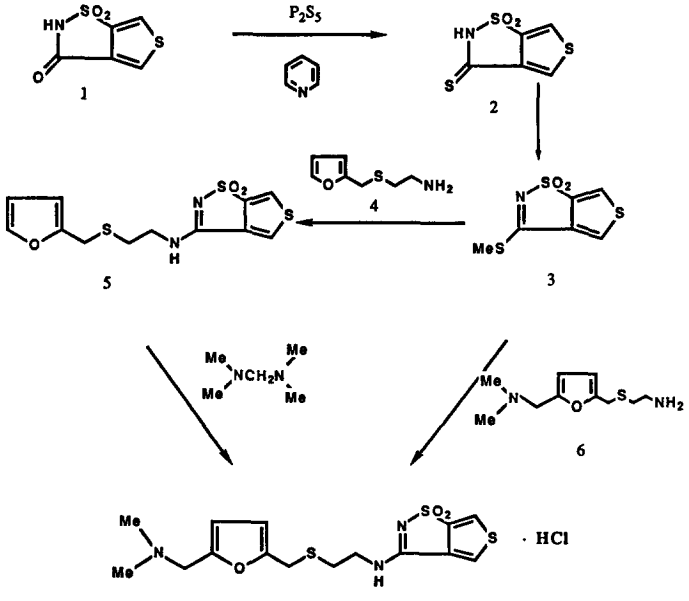
Cimetidine (Tagamet)



Ranitidine (Zantac)

Chemistry

Scheme 1 shows the route to prepare the title compound. Thiation of the previously described **1** [4] with phosphorous pentasulfide in pyridine afforded the thione **2** in 40% yield.



Scheme 1.

Treatment of the sodium salt of **2** with methyl iodide in water gave **3**. Displacement of methyl mercaptan from **3** by an equivalent of 5-(dimethylamino)methyl-2-furanylmethylthioethyl amine [5] **6** in refluxing ethanol gave the free base of **7** which, when treated with a solution of hydrogen chloride in ethanol, gave **7**.

Alternatively, **7** was prepared from **3** in a two-step process by first displacing methylmercaptan in **3** with **4** [6] in refluxing ethanol giving **5**. Treatment of **5** with *N,N,N',N'*-tetramethyldiaminomethane in acetic acid overnight at room temperature followed by the addition of water and neutralization with sodium bicarbonate solution gave the free base of **7**. The IR spectrum was identical with that of the free base of the title compound prepared by the previous route.

Biological results and discussion

Compound **7** evinced H_2-receptor antagonist activity in both *in vitro* and *in vivo* biological assays. In the isolated guinea pig right atria, an *in vitro* assay, **7** induced a rightward shift in the histamine-induced, positive chronotropic dose—response curve. Comparative studies were carried out with the H_2-antagonists cimetidine and ranitidine and representative results are shown in Fig. 1. The rank order of potency, as indicated by their respective pA_2 values, was **7** (pA_2 = 8.23; 8.20—8.27) > ranitidine (pA_2 = 6.91; 6.79—7.02) > cimetidine (pA_2 = 6.40; 6.17—6.62). (The range following each pA_2 value indicates its 95% confidence interval.) The Schild coefficient (± SEM) for **7** was 1.09 (± 0.01).

This *in vitro* activity was confirmed *in vivo* in two species, the dog and the rat. In the dog, **7** *(p.o.)* dose-dependently suppressed the stimulation of gastric acid secretion elicited by consumption of a standardized meat meal. This data is shown in Fig. 2, in comparison with similar data obtained with cimetidine and ranitidine. The greater *in vitro* potency of **7** relative to ranitidine and cimetidine was mirrored *in vivo*, with respective ED_{50} values and associated 95% confidence intervals of 0.20 (0.13—0.27) mg/kg for **7**, 0.93 (0.67—1.25) mg/kg for ranitidine and 6.04 (3.38—8.42) mg/kg for cimetidine. Compound **7** is thus 5-fold more potent than ranitidine and 30-fold more potent than cimetidine in the dog *in vivo* (with ED_{50}s of 0.11 mg/kg for ranitidine and 0.52 mg/kg for **7**). **7** appeared more potent than ranitidine against histamine-stimulated acid secretion as well, though this difference escaped being considered statistically significant because of some variability in the ranitidine data (data not shown). Similar findings were observed when the gastric acid anti-secretory activities of ranitidine, cimetidine and **7** were evaluated in the pylorus-ligated rat. This model, which assesses the ability of compounds under investigation to reduce basal acid secretion, indicated that **7** with an ED_{50} of 0.43 mg/kg (95% confidence interval 0.19—1.13 mg/kg), was significantly more potent than either ranitidine or cimetidine, which had respective ED_{50} values and 95% confidence intervals of 9.97 (6.20—17.8) mg/kg and 18.5 (12.6—27.3) mg/kg. The inhibition expressed by **7** both *in vitro* and *in vivo* was specific to histamine H_2-receptors in that 1) at 1 μM, **7** had no effect on either the carbachol- or the histamine-induced positive inotropic response in the isolated guinea pig ileum, and 2) it had no effect *in vivo* (10 mg/kg, *p.o.*) on either heart rate or blood pressure in the conscious rat or dog.

The absence of the dimethylaminomethyl function from the furan ring as in **5** rendered the compound inactive in the rat (data not shown). This lack of activity in **5** precluded its further testing.

The relevance of preclinical *in vitro* and *in vivo* assays, such as those employed here, as predictors of investigational compounds' potential clinical efficacy is variable, depending

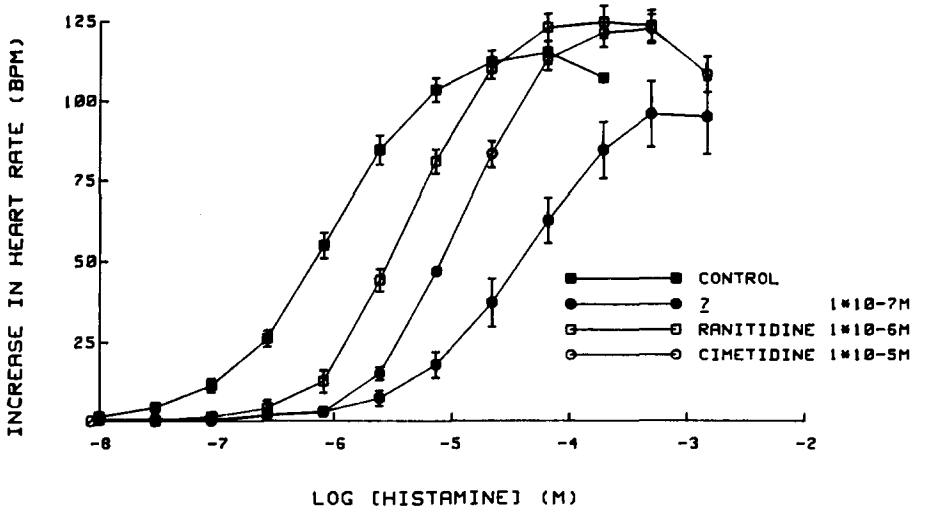


Fig. 1. Effect of **7**, ranitidine or cimetidine on the histaminic positive chronotropic response in isolated guinea pig right atria. Histamine dose—response curves were constructed first in the absence and then in the presence of the H_2-antagonist at the concentrations indicated. Control values (n = 12) from the 3 experiments were pooled. For each experiment, all determinations were carried out in quadruplicate. All data points are means ±SEM. In these representative experiments, the greatest rightward shift in the dose—response curve was elicited by **7**, which was present at a concentration, respectively, 10- and 100-fold lower than those used for ranitidine or cimetidine. Additional shifts were carried out with each of the three H_2-antagonists and, based on these additional data, pA_2 values were calculated as described in the text.

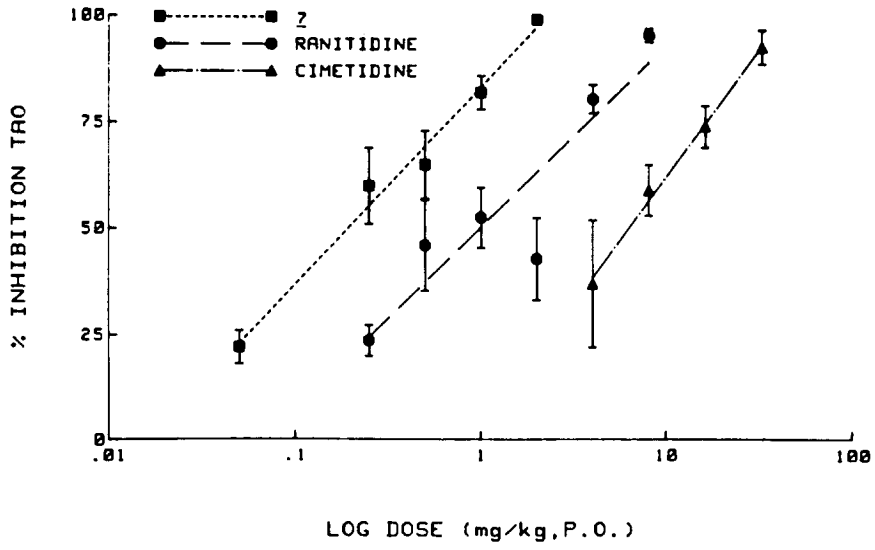


Fig. 2. Inhibition of food-stimulated gastric acid secretion in the innervated gastric pouch dog by **7**, ranitidine or cimetidine. Dogs received, *p.o.*, the drug administered at the dose indicated, or saline, with all results being expressed as percent of control. As described in the text, control values were obtained at each dose and each dog served as its own control. At least 3 days were interspersed between control and drug-treated experiments. Values shown are means ±SEM based upon 3—6 individual determinations. TAO = total acid output.

upon the specific clinical application. In anti-ulcer therapy, there is a strong correlation between the clinical efficacy of compounds, such as ranitidine and cimetidine, and their ability to 1) reduce gastric acid secretion in animal models and 2) act as H_2-receptor antagonists *in vitro*. A further extension of this continuity is now evident with **7**. Thus, **7** has been shown in this present report to possess superior activity in its preclinical evaluation and, moreover, it has also recently been shown clinically to significantly reduce acid secretion in man at doses as low as 10 mg [7—9].

Experimental protocols

Chemistry

Melting points were determined on a Thomas—Hoover apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. NMR spectra were recorded on Varian XL-300 and XL-100 instruments. Mass spectra were recorded with a Kratos MS-25 instrument. IR spectra were recorded with a Perkin—Elmer 299 infrared spectrophotometer. Elemental analyses were performed with a Perkin—Elmer Model 240 elemental analyzer by the Analytical Section of our laboratories and all analyses were within ± 0.4% of theoretical values. Samples of cimetidine and ranitidine were obtained from Smith Kline & French Laboratories and Glaxo, Inc., respectively.

*Thieno[3,4-*d*]isothiazole-3(2*H*)-thione 1,1-dioxide* **2**
To a mixture of 5.6 g (0.03 mol) of thieno [3,4-*d*]isothiazol-3(2H)-one-1, 1-dioxide **1** [4] in 50 ml of dry pyridine was added 5.6 g (0.016 mol) of P_2S_5 portionwise over 3 min. The viscous mixture was heated slowly in an oil bath under an atmosphere of N_2. The temperature of the oil bath was kept at 80°C for 25 min, the internal temperature reading 63°C. The reaction solution was cooled to 50°C and was added dropwise over 5 min to 200 ml of H_2O and cooled in an ice bath. The precipitate which formed was collected and discarded. The filtrate was cooled in ice and acidified with conc HCl to pH 1. The resulting product amounted to 2.5 g (40%). An analytical sample (mp: 196—198°C) was obtained by recrystallization from H_2O. Anal. ($C_5H_3NO_2S_3$) C, H, N.

*3-(Methylthio)thieno[3,4-*d*]isothiazole 1,1-dioxide* **3**
To a mixture of 0.9 g (0.0044 mol) of **2** in 4 ml of EtOH was added a solution of 0.35 g (0.0044 mol) of 50% NaOH in 3 ml of H_2O. To this viscous mixture was added 0.62 g (0.0044 mol) of CH_3I. The mixture was heated under reflux for 5 min and was then filtered and cooled to give 0.45 g (47%) of product. An analytical sample (mp: 184—186°C) was obtained by recrystallization from EtOH. Anal. ($C_6H_5NO_2S_3$) C, H, N. ^{1}H NMR (DMSO-d_6): 2.65 ppm (s, 3, SMe); 8.40 ppm (A, B quartet, 2H, thiophene protons).

N-*[2[[[-5-(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]thieno[3,4-*d*]isothiazol-3-amine 1,1-dioxide, hydrochloride* **7**
To a suspension of 61.92 g (0.28 mol) of **3** in 800 ml of absolute EtOH was added 60.52 g (0.28 mol) of **6**. The mixture was stirred vigorously and was heated at reflux temperature for 2 h. A clear solution was formed in approximately 10 min. The reaction mixture was evaporated to dryness in a rotary evaporator, leaving a solid white mass. The residue was triturated with 600 ml of EtOEt giving a white solid which amounted to 107 g (mp: 106—109°C) after drying (96%). Anal. ($C_{15}H_{19}N_3O_3S_3$ free base) C, H, N. ^{1}H NMR ($CDCl_3$) 2.26 ppm (s, 6H, $N(Me)_2$); 2.80 ppm (t, 2H, SCH_2); 3.42 ppm (s, 2H, CH_2N); 3.48 ppm (q, 2H, CH_2NH); 3.72 ppm (s, 2H, CH_2 furan); 6.45 ppm (A, B quartet, 2H, furan protons); 7.72 ppm (A, B quartet, 2H, thiophene protons).

To a solution of 109 g (0.28 mol) of the free base in 1.5 l of warm absolute EtOH was added sufficient ethanolic HCl solution to obtain a reaction solution of pH 1 (litmus). The yellow solution was cooled in ice and the hydrochloride salt which precipitated was collected on a suction filter. The filter cake was washed with 500 ml of cold absolute EtOH. The product amounted to 118 g (99%; mp: 187°C) after drying in air. The product was dissolved in 15 l of boiling ethanol and then was concentrated to 13 l. After cooling in an ice bath, the crystalline product was collected on a suction filter, rinsed with petroleum ether and dried at 90°C giving 93 g of purified **7** (mp: 189°C; 76% overall yield). Anal. ($C_{15}H_{19}N_3O_3S_3$·HCl) C, H, N. ^{1}H NMR (DMSO-d_6); 2.70 ppm (s, 6H, $N(Me)_2$); 2.88 ppm (t, 2H, CH_2S); 3.55 ppm (q, 2H, CH_2NH); 3.90 ppm (s, 2H, CH_2 furan); 4.35 ppm (s, 2, CH_2N^+); 6.55 ppm (A, B quartet, 2H, furan protons); 8.35 ppm (A, B quartet, 2H, thiophene protons); 10.05 ppm (t, 1H, NH); 10.90 ppm (broad s, 1H, N^+ H).

N-*[2-[(2-Furanylmethyl)thio]ethyl]thieno[3,4-*d*]isothiazol-3-amine 1,1-dioxide* **5**
A stirred mixture of 2.0 g (0.013 mol) of 2-[2-furanylmethyl)thio]-ethanamine **4** [6] and 2.79 g (0.013 mol) of **3** in 50 ml of EtOH was

heated under reflux for 4.5 h. The reaction mixture was filtered giving 1.65 g of a first crop and on cooling the filtrate, 3.94 g of a second crop of product separated (92%). The analytical sample (mp: 146—148°C) was obtained by recrystallization of a portion from EtOH. Anal. ($C_{12}H_{12}N_2O_3S_3$) C, H, N. ^{1}H NMR (DMSO-d_6): 2.70 ppm (t, 2H, SCH_2); 3.50 ppm (t, 2H, CH_2N); 3.85 ppm (s, 2H, CH_2 furan); 6.35 ppm (m, 2H, 3,4 furan protons); 7.60 ppm (s, 1H, 5 furan proton); 8.15 ppm (A, B quartet, 2H, thiophene protons); 9.45 ppm (s, 1, NH).

Preparation of 7 (free base) from 5
To 0.328 g (0.001 mol) of **5** in 10 ml of glacial HOAC was added 0.102 g (0.001 mol) of *N,N,N′,N′*-tetramethyldiaminomethane. The reaction mixture was stirred at ambient temperature overnight and was then poured into 200 ml of H_2O. The solution was basified by the careful addition of K_2CO_3 until the evolution of CO_2 ceased. Upon cooling the mixture in ice, 0.318 g of **7** (free base) were obtained (82%; mp: 95—98°C). The IR spectrum of this product was identical with that of **7** prepared *via* the reaction of **3** with **6**. Anal. ($C_{15}H_{19}N_3O_3S_3$) C, H, N.

Pharmacology

Pylorus-ligated rat
Basal acid secretion in the rat was determined according to the method of Shay *et al.* [10]. Male 190—260 g Charles River rats (strain SD/CD) were fasted for 24 h with access to tap water *ad libitum* until the test. Groups of 10 rats each were assigned to either control or drug treatment. Each dose—response experiment consisted of a minimum of 3 treatment groups and one control group (*i.e.*, $n = 40$). Under methohexital anesthesia (40 mg/kg), a midline laparotomy was performed and a ligature tightly secured around the pylorus. Either control vehicle (0.25% aqueous methylcellulose) or drug in control vehicle was administered intraduodenally (1 ml/kg), immediately after ligating the pylorus. The abdominal incision was closed, the rats were allowed to recover from anesthesia and then they were sacrificed by CO_2 asphyxiation 4 h later.

The volume of gastric juice was recorded and the acid concentration of 1.0 ml sample aliquots was measured by electrometric titration to pH 7.0 using 0.1 N sodium hydroxide. The product of the gastric volume and acid concentration was used to calculate the total acid output. Total acid output after drug administration was compared with that obtained in control animals and results expressed as percent inhibition. Any samples having coprophagic contamination were excluded.

Thomas modification of the Pavlov—pouch dog
Food- or histamine-stimulated acid secretion in the dog was determined using dogs prepared with modified Pavlov—pouches as described by Thomas [11]. Female beagles weighing 9—13 kg were surgically prepared with innervated gastric pouches and allowed a recovery period of at least 2 weeks. The dogs were fasted for 18 h with access to tap water *ad libitum* until the test. Two samples of gastric secretions were collected at 15 min intervals to establish a baseline. The control vehicle (0.9% saline) or drug in control vehicle was administered by oral gavage and 2 additional 15 min samples were taken. 30 min after treatment, the dogs were given a 200 ml portion (185 g) of commercially-prepared, 100% meat meal. Dogs routinely consumed the entire meal within 5 min and any dog not consuming the entire meal within 15 min was excluded from the test. After feeding, gastric pouch samples were collected at 15 min intervals until the test was terminated 4 h later.

When histamine was used as the secretagogue, drug or vehicle, as indicated above, was administered by oral gavage 30 min prior to administration of histamine diphosphate in saline (64 μg/kg histamine base, s.c.). Two samples at 15 min intervals were collected during this time period. For the duration of the action studies, drug or vehicle was administered *(p.o.)* the indicated number of hours plus 30 min before histamine.

The volume of secreted gastric juice was recorded and the acid concentration of 1.0 ml aliquots was measured by electrometric titration to pH 7.0 using 0.1 N sodium hydroxide.

The product of the gastric volume and acid concentration was used to calculate the total acid output (TAO). A mean ± SE value was calculated for each time period and the sum, over a 3.25 h time period for experiments involving food stimulation or over a 1 h time period for experiments involving histamine stimulation, was calculated to provide the TAO. Total acid output after drug administration was compared with that after saline (control value) and results expressed as the percent inhibition. Routinely, the number of determinations carried out at each dose of drug was equal to 6, with the minimum number of determinations never being less than 3. At each drug dose, the mean percent inhibition value ± SEM was calculated from the individual values.

Isolated guinea pig right atria
Isolated guinea pig right atria were prepared as described by Black *et al.* [12] Male guinea pigs from Charles River weighing 250—325 g were sacrificed by cervical dislocation. The right atria were dissected free and suspended in 10 ml isolated tissue baths under a 1 g tension load. Contraction rates were monitored *via* a Grass Model FT03 force displacement transducer. Krebs—Henseleit buffer of the following composition was used: 117.5 mM NaCl; 1 mM $NaH_2PO_4 \cdot H_2O$; 5.4 mM KCl; 25 mM $NaHCO_3$; 2.5 mM $CaCl_2$; 11.1 mM glucose. The Krebs-Henseleit bath solution was oxygenated (95—5%; O_2—CO_2) and maintained at 32°C. After a 1 h equilibration period, cumulative histamine dose—response curves were carried out. The tissues were then washed, the heart rate allowed to return to basal level and then, following addition of the histamine H_2-receptor antagonist to each tissue and a 30 min equilibration period, the histamine dose—response curve was repeated. Regression analysis of the log (dose ratio—1) *versus* the log of the antagonist concentration gave the intercept and slope with 95% confidence limits. The H_2-receptor antagonist potency was reported as the pA_2 value, *i.e.*, the negative log molar concentration that produced a dose ratio of two.

Acknowledgments

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