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Original paper

Potential histamine H_2-receptor antagonists: synthesis and pharmacological activity of derivatives containing 3-alkylamino-4-aminofurazan moieties**

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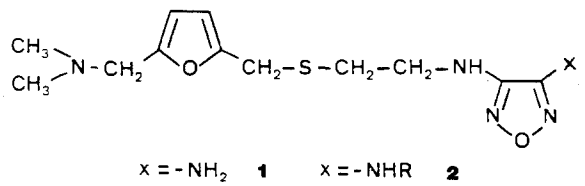
Summary — A series of 3-alkylamino-4-(2-((5-dimethylaminomethyl-2-furyl)methylthio)ethylamino)furazans were prepared and tested for their H_2-antagonist activities on guinea pig right atrium. A number of differently shaped alkyl substituents on the terminal 3-amino group were favourable for activity. The most potent compound was the cyclohexylmethyl-substituted derivative (pA_2=8.83). Most compounds showed non-competitive antagonism at histamine H_1 and muscarinic receptors at concentrations approximately 100 times higher than those producing competitive H_2-receptor block. This finding suggests that there is an accessorial binding area on H_2-receptor near the site fitted by the diaminofurazan moiety.

Résumé — **Antagonistes potentiels du récepteur H_2 de l'histamine: synthèse et activité pharmacologique de dérivés porteurs de restes alkylamino-3 amino-4 furazane.** *On a préparé une série d'alkylamino-3-(((diméthylaminomethyl-5-furyl-2)méthylthio)-2-éthylamino)-4 furazanes. Les produits ont été essayés comme antagonistes de la tachycardie stimulée par l'histamine dans l'oreillette droite du Cobaye* in vitro. *La plupart des groupes alkyles positionnés sur le substituant aminé en 3- favorisent l'activité. Le produit portant le groupe cyclohexylméthyle est le plus actif de la série (pA_2=8.83). Les produits montrent une bonne sélectivité pour les récepteurs H_2. Ces résultats semblent montrer l'existence sur le récepteur H_2 d'un site de liaison supplémentaire adjacent au site occupé par le groupement diaminofurazanique.*

H_2-antihistaminics / alkylaminofurazan / H_2-receptor antagonists / H_2-receptor accessorial binding area

Introduction

In preceding papers [1, 2] we reported the synthesis and H_2-antagonist activity of some derivatives related to ranitidine containing, as neutral polar substructure, the aminofurazan system. The 3-amino-4-(2-((5-dimethylaminomethyl-2-furyl)methylthio)ethylamino)furazan **1** (pA_2 =7.43) [1] was as potent as ranitidine and did not display any activity on β-adrenergic, H_1 and muscarinic receptors at concentration 10^{-5} M.



X = -NH2 **1** X = -NHR **2**

The replacement in this model of the 3-amino group with a methyl or a phenyl substituent afforded a selective but less potent antagonist (pA_2=6.19) and an inactive compound, respectively [2]. The decrease in activity in the methyl analogue was attributed to a lesser ability of this model to interact by hydrogen bonds with the receptor, while the inactivity of the phenyl derivative was thought to be due principally to steric factors.

These results prompted us to gain more insight into the structure–activity relationships in this class of compounds. In the present paper we report synthesis and results of an evaluation as H_2-antagonists of a series of derivatives with structure **2** in which R is a differently shaped alkyl group. Such a molecular manipulation has the purpose of increasing the affinity of the drug for the receptor through hydrophobic or van der Waals interactions with possible non-polar accessorial binding area adjacent to the site fitted by the diaminofurazan system [3].

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***This work is dedicated to Prof. Guido Tappi on the occasion of his 75th birthday.*

So far as the studies on H_2 receptor are concerned, a similar approach was tried without reaching definitive conclusions by Emmett *et al.* working with a series of benzylhistamines [4]. Recently also Schunack and co-workers related activity to lipophilicity in a series of cimetidine analogues containing alkyl-substituted pyrimidone moieties [5].

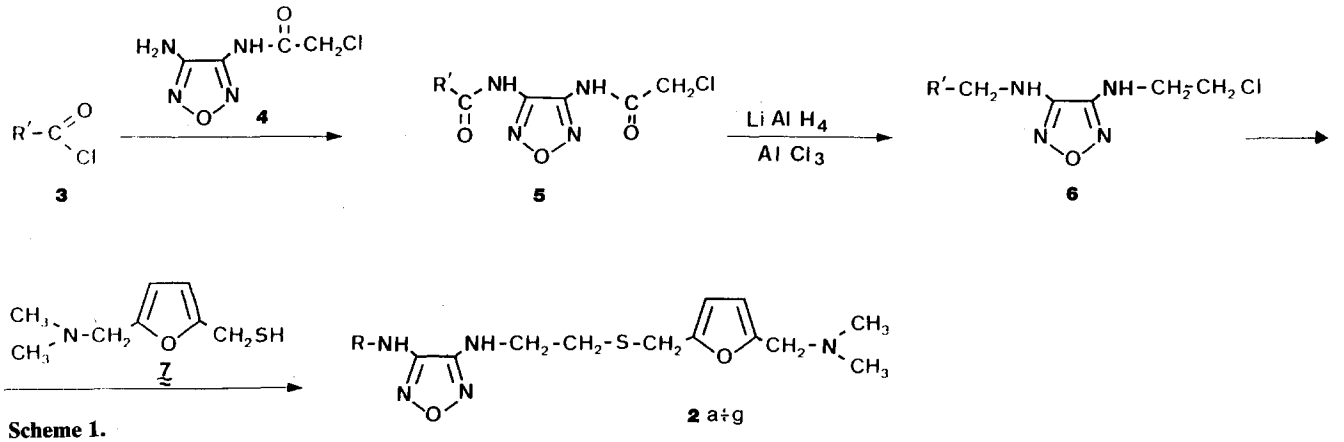
Chemistry

The synthetic pathway used to obtain compounds **2** (see Table I; for the intermediates see Table II) is reported in Scheme 1.

The appropriate acylchlorides **3** were allowed to react with 3-amino-4-chloroacetamidofurazan **4** [1] to afford the *bis*-acylamino derivatives **5**. Reduction of these intermediates with a mixture of $LiAlH_4 / AlCl_3$ produced the *bis*-alkylamino derivatives **6** which by reaction with nucleophile **7** [6] gave the final compounds **2**. ^{13}C characterization of the products **2** is reported in Table III.

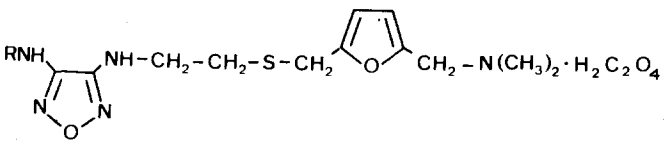
Pharmacology

2 a–g Oxalates were tested as antagonists of the positive chronotropic action of histamine in the isolated guinea pig atria according to the procedure described by Black *et al.* [7]. None of the compounds produced modification of the spontaneous contraction frequency when tested alone. The activity expressed as $-\log K_B$ (pA_2) is reported in Table I. Specificity for H_2-receptors was evaluated on the



Scheme 1.

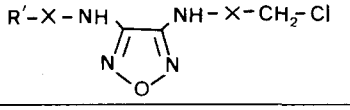
Table I. Reaction yields, melting points and H_2-antagonistic activity of **2**.



Compound	R	Yield (%)	Mp (°C)	$pA_2 \pm 95\%$ CL	Slope of Schild plot ± 95% CL	No. of experiments
2a	CH_2-CH_3	65	145–146[a,b]	7.34 0.32	0.89 0.23	16
2b	$CH_2-CH_2-CH_3$	67	159–160	8.10 0.19	1.05 0.18	20
2c	$CH_2-CH(CH_3)_2$	60	151–152[b]	8.22 0.20	1.09 0.18	19
2d	$CH_2-C(CH_3)_3$	70	149–150[b]	7.68 0.19	1.11 0.17	18
2e	$CH_2-C_6H_5$	75	127–128[b]	7.65 0.21	1.10 0.18	21
2f	$(CH_2)_3-C_6H_5$	70	124–125[b]	8.69 0.23	1.04 0.19	21
2g	$CH_2-C_6H_{11}$	67	144–145	8.82 0.18	1.05 0.16	20
1	H	c	c	7.43 0.33[c,d]	c	c

[a]With decomposition.
[b]After initial softening.
[c]See [1].
[d]The pA_2 value of this compound has been periodically controlled without finding any significant variation.

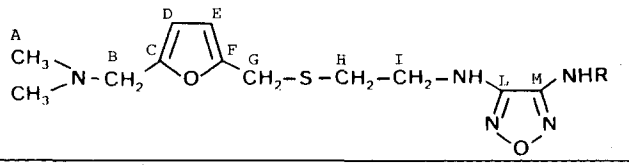
Table II. 3-Acylamino-4-(2-chloroacetamido)furazans **5** and 3-alkylamino-4-(2-chloroethylamino)furazans **6**.



No.	R′	X	Yield (%)	mp (°C)	Formula
5a	CH_3	CO	60	152–153	$C_6H_7ClN_4O_3$
5b	CH_2CH_3	CO	80	134–135	$C_7H_9ClN_4O_3$
5c	$CH(CH_3)_2$	CO	75	122–123	$C_8H_{11}ClN_4O_3$
5d	$C(CH_3)_3$	CO	60	136–137	$C_9H_{13}ClN_4O_3$
5e	C_6H_5	CO	60	154–155	$C_{11}H_9ClN_4O_3$
5f	$(CH_2)_2C_6H_5$	CO	70	147–148	$C_{13}H_{13}ClN_4O_3$
5g	C_6H_{11}	CO	75	142–143	$C_{11}H_{15}ClN_4O_3$
6a	CH_3	CH_2	75	a	$C_6H_{11}ClN_4O$
6b	CH_2CH_3	CH_2	70	a	$C_7H_{13}ClN_4O$
6c	$CH(CH_3)_2$	CH_2	72	a	$C_8H_{15}ClN_4O$[b]
6d	$C(CH_3)_3$	CH_2	65	79– 80[c]	$C_9H_{17}ClN_4O$
6e	C_6H_5	CH_2	73	80– 81[c]	$C_{11}H_{13}ClN_4O$
6f	$(CH_2)_2C_6H_5$	CH_2	71	56– 57[c]	$C_{13}H_{17}ClN_4O$
6g	C_6H_{11}	CH_2	72	63– 64[c]	$C_{11}H_{19}ClN_4O$

[a]Oil.
[b]H: Req'd 6.91; found 7.50.
[c]Recrystallization: **6d** from a mixture benzene and cycloexane; **6e** from petroleum benzine 40°/60°; **6f** and **6g** from a mixture petroleum benzine 40°/60° and ethyl acetate.

Table III. ^{13}C NMR data of derivatives **2a–g**.



	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
A	44.85	44.47	44.18	44.44	43.98	44.32	44.45
B	55.80	55.46	55.20	55.45	54.99	55.30	55.44
C,F*	151.44,149.59	151.73,149.74	151.99,149.66	151.68,149.64	150.19,149.70	151.78,149.70	151.49,149.66
D,E	109.80,108.13	110.54,108.33	110.93,108.33	110.54,108.33	110.93,108.37	110.70,108.33	110.38,108.30
G	28.35	28.66	28.78	28.69	28.61	28.64	28.64
H	31.07	31.32	31.48	31.24	31.19	31.28	31.29
I	43.17	43.40	43.30	43.48	43.40	44.25	43.32
L,M*	151.15,149.98	150.63,150.34	150.47,150.04	151.07,150.61	151.88,149.88	150.34,150.22	150.71,150.45
R	39.41, 14.40	46.56, 22.38 11.42	52.45, 27.91 20.24	56.70, 32.10 27.33	138.20,128.54, 127.91,127.50, 48.64	141.50,128.38, 128.43,125.96, 43.33, 33.18, 30.59	51.15, 37.22, 30.91, 26.43, 25.88

*Assignment tentatives.

basis of the ability of these compounds to antagonize the chronotropic response to isoprenaline in the guinea-pig atrium (β-adrenergic receptors) and the contractile response to histamine and carbachol in the guinea pig ileum (H_1 and muscarinic receptors, respectively); the compounds were tested according to the technique reported previously [8].

Results and Discussion

The data reported in Table I show that the introduction of an alkyl moiety on the lateral 3-amino substituent in **1** is always accompanied by maintenance (**2a**, **2d**, **2e**) or by an increase (**2b**, **2c**, **2f**, **2g**) of the H_2-antagonistic activity with respect to the starting lead. Therefore, we think that near the H_2-receptor area fitted by the diaminofurazan substructure, a space where the alkyl substitutents fall, extending for a distance sufficient to accommodate the phenylpropyl group, has to exist. On the basis of the present data it is impossible to utilise a reliable QSAR approach to clarify whether this region is sterically constrained or unconstrained, if the binding of the alkyl substituents is truly hydrophobic in character and if they exert a buttressing action which helps to hold the molecules in place in the complex with the receptor.

The problem of the selectivity of the compounds **2** is worthy of comment. None of them modified the positive chronotropic effect of isoprenaline on guinea pig atrium. On the contrary, all the derivatives non-competitively antagonized the contractile response to histamine and carbachol on the guinea pig ileum, with only the partial exception of **2a** and **2b**, which did not influence the effect of carbachol. This noncompetitive antagonism arose at concentrations $\approx$100 times higher than those producing competitive H_2-receptor block.

In conclusion, these results suggest that on H_2receptor, near the site fitted by the cyclic "urea equivalent" group, there is an accessorial binding area. This finding opens the possibility of designing new compounds with high H_2-antagonistic activity.

Experimental protocols

Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. All derivatives were routinely checked by IR (Perkin–Elmer 781), ^{1}H NMR, ^{13}C NMR (Jeol GX 270/89) and mass (Varian CH7 MAT) spectrometry to confirm the proposed structures. Microanalyses for C, H, N are within $\pm$0.4% of the theoretical values. Merck silica gel Kieselgel 60, 230–400 mesh ASTM was employed for the chromatographic purifications.

3-Acylamino-4-(2-chloroacetamido)furazans ***5***
To a stirred solution of **4** (2.0 g, 0.011 mol) in dry THF (40 ml) 0.022 mol of the appropriate acyl chloride were added. The solution was refluxed for 2 h and, after addition of sodium bicarbonate (1.85 g, 0.022 mol), was evaporated under vacuum. The residue, treated with water, was extracted with ethyl acetate. After drying ($MgSO_4$) the solvent was removed under reduced pressure and the solid thus obtained was recrystallized from petroleum benzine 40°/60° and ethyl acetate. In the case of **5d** and **5g**, the crude products were filtered on a short silica gel column (eluent, chloroform:ethyl acetate 9:1) before crystallization. Reaction yields and melting points are reported in Table II.

3-Alkylamino-4-(2-chloroethylamino)furazans ***6***
To stirred and ice-cooled mixture of dry THF (45 ml) and lithium aluminium hydride (0.69 g, 0.018 mol), aluminium chloride (1.22 g, 0.0091 mol) was added portion-wise. The stirring and cooling were continued for 40 min and then the appropriate *bis*-acylamino derivative **5** (0.0046 mol) was added portion-wise under ice-salt refrigeration. After 2 h the reaction mixture was treated with ethyl acetate and then with 10% sulfuric acid. The separated organic phase was stirred for 30 min on anhydrous sodium carbonate powder, filtered and evaporated under vacuum. The oily residue was purified by siliga gel column chromatography (eluent, petroleum benzine 40°/60°:ethyl acetate 9:1). The purified products were obtained as oils. Some of them slowly changed into colourless solids on standing. Reaction yields, crystallization solvents, and melting points are reported in Table II.

3-Alkylamino-4-[2-[(5-dimethylaminomethyl-2-furyl)methylthio]ethylamino]furazans ***2***
To a stirred and ice-water cooled solution of sodium (0.09 g, 0.0040 mol) in the ethyl alcohol (10 ml), **7**. HCl (0.42 g, 0.0020 mol) was added portion-wise under nitrogen. The appropriate bis-alkylaminofurazan **6** (0.0018 mol) dissolved in ethyl alcohol (2 ml) was added. The mixture was kept under stirring and cooling for 10 min and then refluxed for 1 h. The solvent was removed under vacuum and the residue was treated with water and extracted with ethyl acetate. The organic layer, dried on $MgSO_4$, was evaporated under reduced pressure. The oils thus obtained were purified by silica gel column chromatography (**2a–c**, **e**, eluent, chloroform:methanol 95:5; **2d**, **f**, **g**, eluent, chloroform:methanol, 99:1) and immediately transformed into the corresponding oxalates. Reaction yields, melting points of the oxalates after recrystallization from a mixture of isopropanol/methanol are reported in Table I.

Acknowledgments

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