Potential Histamine H_2-Receptor Antagonists: Analogues of Classical Antagonists Containing 4-Substituted-3-Aminofurazan Moieties

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Analogues of classical H_2-receptor antagonists containing aminofurazan moieties as polar groups were synthesized and tested „in vitro" for their activities. Compounds containing 3-amino-4-methylfurazan and 4-amino-3-methylfuroxan substructures show good antagonistic properties. The compound containing the 3-amino-4-phenylfurazan moiety is inactive. An SAR approach is discussed.

Potentielle Histamin H_2-Rezeptor-Antagonisten: Analoge klassischer Antagonisten mit 3-Amino-4-substituierten-Furazan-Gruppen
Einige den klassischen H_2-Antagonisten ähnliche Produkte, die als polare Gruppen Aminofurazan-Einheiten enthalten, wurden hergestellt und auf ihre „in vitro" Wirkung geprüft. Die die 3-Amino-4-methylfurazan- und die 4-Amino-3-methylfuroxan-Gruppen enthaltenden Produkte zeigten gute H_2-antihistaminischen Eigenschaften. Das Produkt, das die 3-amino-4-phenylfurazan-Einheit enthält, war inaktiv. Struktur-Wirkungs-Relationen werden diskutiert.

In an earlier paper[1] we demonstrated that a diaminofurazan substructure can be used as "urea equivalent" group to design new potent analogues of classical H_2-antagonists. Consequently this substructure must have a high affinity for that H_2-receptor area which binds. This statement is further supported by the finding that it is possible to obtain H_2-antagonists with a fairly good activity in which this moiety is joined by a flexible chain to aminomethyl-substituted aliphatic systems[2]. In order to investigate the possibility of using 3-amino-4-methylfurazan **(1)**, its 3-methyl N-oxide **3** and 3-amino-4-phenylfurazan **(2)** as polar groups for the design of new H_2-antagonists we synthetized and screened for their "in vitro" H_2-activity compounds **1e, f, 2e, f** and **3a**.

These substructures contain only one N-H group, analogously to the moieties present, for example, in Hoe 062 and in Hoe 760[3], in Wy 45086, in famotidine and in other anti-H_2 drugs[4].

Table 1: ***Physico-chemical data of*** **1, 2, 3**

	1	2	3
pK_a (proton lost) at 25 C°	>11[a]	>11[a]	>11[a]
pK_a (proton gain) at 25 C°	−2.15[b]	−2.31[c]	−3.01[d]
log P (oct/H_2O)	0.087	1.81	−0.17

a) Upper limit of potentiometric method with glass electrodes.
b) Taken from ref. 7.
c) Calculated from the equation $pK_a = -2.20 - 2.72\ \sigma_m^o$ using for $C_6H_5\ \sigma_m^o = 0.04$[8].
d) See text.

Physical-Chemistry and Chemistry

Compounds **1, 2** and **3** show amphoteric and hydrophilic properties in keeping with those of other polar groups present in classical H_2-antagonists (Table 1). In addition X-ray studies have demonstrated that **1**[5] and **3**[6] are structures in which the five membered rings are planar and the NH_2 groups are only a little rotated in comparison with the heterocyclic rings.

The behaviour to the protonation of **3** deserves some comments. This compound is a very weak base and its pK_a (proton gain) must be determined in strongly acid solutions (Figure 1).

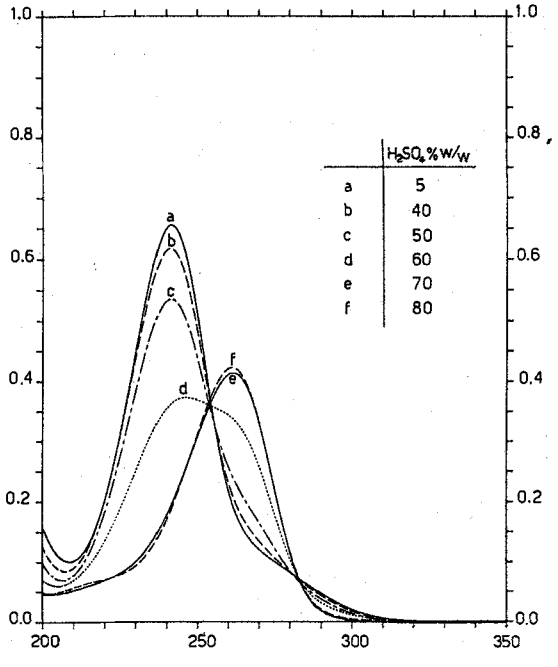

Figure 1: UV spectra of 3 in sulphuric acid at different concentrations

Protonation does not satisfy equation 1) with m = 1 and so **3** is not a *Hammett* base. Moreover protonation is not correlated satisfactorily by other acidity function.

$$\log I = -m\ Ho + pK_a \qquad 1)$$

pK_a and m values determined from equation 1) were $pK_a = -3.01$, m = 0.75. Ionization ratios of **3** used for the calculations are reported in Table 2.

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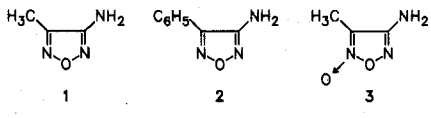
Table 2: *Ionization ratios of 4-amino-3-methylfuroxan* **(3)**

Sulphuric acid (w/w)	log [cation]/[base] (log I)	Sulphuric acid (w/w)	log [cation]/[base] (log I)
49.1	−0.52	57.35	+0.10
52.5	−0.28	59.35	+0.26
53.9	−0.19	61.4	+0.49
56.5	+0.049	64.2	+0.70



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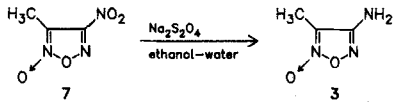
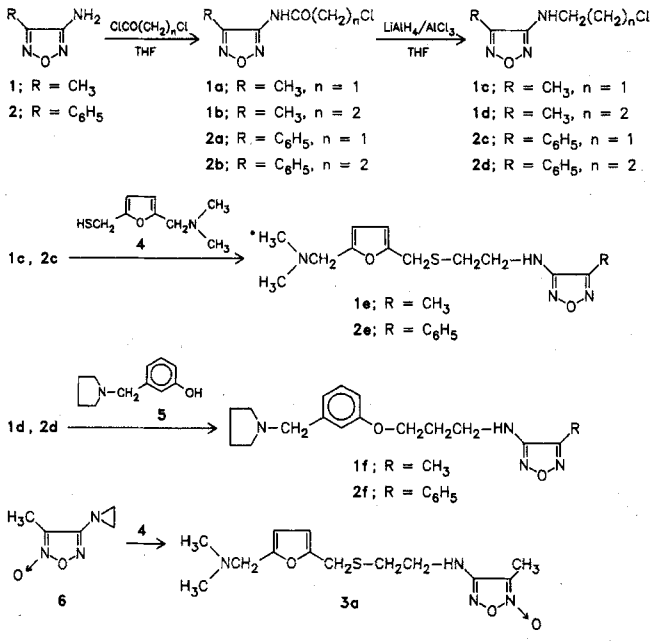


The synthetic routes used for the preparations of **1e, f, 2e, f** and **3a** are reported in Scheme 1



The chloroacylaminofurazans **1a, b** and **2a, b** obtained by action of the appropriate chloroacyl chlorides on the parent aminofurazans were reduced with 2:1 mixture of $LiAlH_4$ and $AlCl_3$ in anhydrous THF to give the expected chloroalkylaminofurazans **1c, d, 2c, d**. This reaction was central for our synthetic goals because the aminofurazans **1** and **2** appeared inert to react with chloroalkyl derivatives under several reaction conditions. Standard procedures were used to obtain the final products from these intermediates.

The furoxan model **3a** was prepared in good yield by condensing 4-aziridino-3-methylfuroxan **(6)** with (5-dimethylaminomethyl-2-furyl)methanethiol **(4)** under N_2 in EtOH.

4-Amino-3-methylfuroxan **(3)** used for the physicochemical characterization was obtained by selective reduction of 3-methyl-4-nitrofuroxan **(7)**.

3 Is a white compound (mp 135 °C) which, as expected, does not tautomerize to the 4-methyl isomer by heating. Its spectroscopic behaviour (IR, NMR, MS) is in agreement with the proposed structure. So the report[10] that yellow crystals (mp 175 °C) of 4-amino-3-methylfuroxan can be obtained by catalytic reduction of **7** with H_2 at 100 atm in acetic acid is incorrect.

Table 3: *Physical and analytical data of the new compounds*

Compound	MP, °C	Solvent of crystallization	Molecular formula	Analysis %[a] Calculated			Found		
				C	H	N	C	H	N
1a	107–108 lit.[9] 106–107	ethanol	$C_5H_6N_3O_2Cl$	34.2	3.45	23.9	34.3	3.5	23.9
1b	144–145	water with a few drops of ethanol	$C_6H_8N_3O_2Cl$	38.0	4.3	22.2	38.2	4.2	22.15
2a	154–155 lit.[9] 153–154	benzene	$C_{10}H_8N_3O_2Cl$	50.5	3.4	17.7	50.8	3.3	17.9
2b	158–159	ethanol/water	$C_{11}H_{10}N_3O_2Cl$	52.5	4.0	16.7	52.6	4.0	16.9
1c	48–49	water	$C_5H_8N_3OCl$	37.2	5.0	26.0	37.2	4.9	25.6
1d	68–69	water with a few drops of ethanol	$C_6H_{10}N_3OCl$	41.0	5.75	24.0	41.2	5.8	23.95
2c	60–61	etanol/water	$C_{10}H_{10}N_3OCl$	53.5	4.5	18.7	53.8	4.5	18.9
2d	47–48	cycloexane	$C_{11}H_{12}N_3OCl$	55.6	5.1	17.7	55.7	5.1	17.7
1e.$H_2C_2O_4$	141–142 dec.	ethanol/ethyl ether	$C_{15}H_{22}N_4O_6S$	46.6	5.75	14.5	46.5	5.8	14.3
2e.$H_2C_2O_4$	124–125 dec.	ethanol/ethyl ether	$C_{20}H_{24}N_4O_6S$	59.6	5.4	12.5	59.4	5.3	12.5
1f.$H_2C_2O_4$	128–129	ethanol/ethyl ether	$C_{19}H_{24}N_4O_6$	56.4.	6.0	13.9	56.1	6.4	14.0
2f.$H_2C_2O_4$	171–172 dec.	ethanol	$C_{24}H_{28}N_4O_6$	61.5	6.0	12.0	61.4	5.8	11.9
3	135	ethyl acetate/petroleum ether	$C_3H_5N_3O_2$	31.3	4.4	36.5	31.2	4.4	36.4
3a.$H_2C_2O_4$	128–130	ethanol/ethyl ether	$C_{15}H_{22}N_4O_7S$	44.8	5.5	13.9	45.0	5.7	14.0

[a] Microanalyses were performed by the Micronalytical Service of the Organic and Industrial Chemistry Department, Milan University.

Physical and analytical data for the new compounds are reported in Table 3.

Pharmacology

The activity of **1e, f, 2e, f** and **3a** oxalates as histamine H_2-receptor antagonists was evaluated on guinea-pig isolated atria according to the technique reported previously[11]. None of the compounds, with the sole exception of **2f**, produced modifications of the spontaneous contraction frequency when tested alone. pA_2 values, calculated with the usual procedures, are reported in Table 4.

Table 4: *H_2-receptor activity of derivatives* **1e, 1f; 2e, f; 3a.**

Compound	pA_2 (∓ 95 % CL)	Slope of Schild plot (∓ 95 % CL)	Number of experiments
1e	6.19 ∓ 0.20	1.05 ∓ 0.17	22
1f	7.18 ∓ 0.30	1.04 ∓ 0.23	20
2e	<4.00	–	5
2f[a]	–	–	6
3a	5.84 ∓ 0.12	0.94 ∓ 0.10	16

[a] This compound displays a negative chronotropic effect

None of the compounds modified the positive chronotropic effect of isoprenaline on guinea-pig atrium, nor did they affect the response of guinea-pig ileum to histamine or carbamoylcholine (concentration $2 \cdot 10^{-5}$ and $5 \cdot 10^{-5}$, respectively).

Discussion: Approach to a Structure-Activity-Relation Study.

Table 4 shows that **1** and **3** can be used as polar groups in the design of new H_2-antagonists. In fact **1e, 1f** and **3a** are selective and competitive H_2-antagonists of histamine with a fairly good activity. Neverthless they are less active than the parent compounds in which the diaminofurazan system is present (when in **1e**, R=NH_2, $pA_2 = 7.43$; when in **1f**, R=NH_2, $pA_2 = 8.41$[1]). It means that in these series of derivatives 3-amino-4-methylfurazan **(1)** and its N → O **3** are only partial bioisosters of the 3,4-diaminofurazan. Obviously with these moieties an interaction with the receptor (probably a hydrogen bond interaction) is partially lost. The inactivity of **2e** could be due to steric factors[12]. So while the amino-methylfurazan substructure is able to fit the receptor site, the amino-phenylfurazan substructure is unable to do so.

In conclusion when the furazan system is used as polar group in ranitidine and lamtidine analogues it does not need to bring two NH groups to provide compounds with a good level of activity.

Experimental Part

MP: capillary apparatus, uncorr. IR (Perkin Elmer 781); ^{1}H-NMR (Varian T-60); mass spectrometry (Varian CH7 MAT): the spectra are in accordance with the proposed structures.

Compounds **1**[13], **2**[14], **4**[11], **5**[15], **6**[16], **7**[17] were synthetized according to methods in literature. Silica gel Merck Kieselgel 60, 230–400 mesh ASTM was employed for chromatographic purifications. Physical and analytical: Table 3.

The partition coefficients in (Table 2), were determined by shake-flask method at room temp., using octanol as lipid phase and water as hydrophilic phase. Log P values are the average of four determinations made with different concentrations of solute; the standard deviations are ∓ 0.01.

pK_a of **3** was determined by a spectroscopic method at 25 (∓ 0.1) °C using a Perkin Elmer Lambda 5 UV/VIS spectrophotometer, working at 242 nm. Stock solutions $7 \cdot 10^{-4}$ M in 5 % w/w sulphuric acid of **3** were prepared. 5 ml of these solutions were diluted at 50 ml with sulphuric acid of appropriate concentrations. Weighed amounts of final solutions were standardized, after dilution, by titration using 0.5 N NaOH. Ho values for each solution were obtained by interpolation from the figures tabulated by *Paul* and *Long*[18] which over the considered range are equivalent to those tabulated by *Jorgenson* and *Hartter*[19].

4-Amino-3-methylfuroxan **(3)**

To a stirred and ice cooled solution of 0.725 g (5.00 mmol) of **7** in 50 ml ethanol, a solution of 2.61 g (15.0 mmol) of $Na_2S_2O_4$ in 15 ml water was added in three portions over 2 min. The solution was filtered to remove the insoluble, partially concentrated under vacuum and extracted with ethyl acetate. The org. layers, dried on $MgSO_4$, were evaporated in vacuo. The crude product was purified by flash chromatography (chloroform:methanol, 98:2) to obtain white crystals (yield 20 %).

3-(Chloroacylamino)-4-methylfurazans **(1a)** *and* **(1b)**

These compounds were synthetized starting from **1** and the appropriate chloroacyl chloride following the method reported[1] for the preparation of amino analogues. The reaction solution was evaporated under vacuum and the residue was dissolved in the minimal amount of boiling water. After cooling the title products were obtained. Crystallization from water containing a small quantity of ethanol afforded pure compounds (yield **1a**, 77 %; **1b**, 81 %).

3-(Chloroacylamino)-4-phenylfurazans **(2a)** *and* **(2b)**

To a stirred and ice cooled solution of 1.28 g (7.95 mmol) **2** in a mixture of 16 ml dry benzene and 4 ml dry ether, at first 0.70 ml of dry pyridine and then dropwise 0.65 ml (7.95 mmol) of chloroacetyl chloride dissolved in 7.0 ml of dry ether were added. The reaction mixture was kept under stirring at 0 °C for 30 min and then at room temp. for 3 h. Preparation of **2b** required a slightly different reaction conditions: 3-chloropropionyl chloride was added at room temp. and after 30 min the reaction mixture was boiled for 1.5 h. The reaction mixtures were evaporated under vacuum and the residues were dissolved in boiling aqueous ethanol. After cooling the title products were obtained. Crystallization from aqueous ethanol afforded pure compounds (yield **2a**, 72 %; **2b**, 60 %).

3-(Chloroalkylamino)-4-methylfurazans **(1c), (1d)** *and 3-(chloroalkylamino)-4-phenylfurazans* **(2c), (2d)**

These compounds were synthetized by reduction of **1a, b** and **2a, b** with $LiAlH_4/AlCl_3$ (2:1) mixture, following the procedure reported for the preparation of 4-amino analogues[1], only with the exception that the reaction mixtures were kept at room temp. for 90 min in the preparations of **1c** and **1d** and for 1 h, under ice salt cooling, in the preparations of **2c** and **2d**. The crude erivatives **1c** and **1d** were dissolved in boiling water while **2c** and **2d** in boiling aqueous ethanol. After cooling the pure products were obtained (yield **1c**, 85 %; **1d**, 80 %; **2c**, 80 %, **2d**, 83 %). An analytical sample of **2d** before crystallization was filtered on a short silica gel column (petroleum ether 40–60 °C: ethyl acetate 90:10).

3-(2-((5-Dimethylaminomethyl-2-furyl)methylthio)ethylamino)-4-methyl and 4-phenylfurazan **(1e)** *and* **(2e)**

These compounds were synthetized starting from **1c, 2c** and **4** according to the procedures reported[1] for the preparation of amino analogues. The

crude products were purified by flash chromatography obtaining oils which were immediately transformed into the corresponding oxalates. **1e**: eluent chloroform containing 0–5 % methanol, yield 73 %. **2e**: eluent chloroform:methanol, 97:3, yield 67 %.

3-Methyl and 3-phenyl-4-(3-(3-(pyrrolidinomethyl)phenoxy)propylamino)furazans **(1f)** *and* **(2f)**

These compounds were synthetized starting from **1d**, **2d** and **5** according to the procedure reported[1] for the preparation of amino analogues. The reaction times were 1 d for **1f** and two d for **2f**. The crude products were purified by flash chromatography obtaining oils which were immediately transformed into the corresponding oxalates. **1f**: eluent chloroform containing 0–5 % methanol, yield 63 %. **2f**: eluent chloroform:methanol, 97:3, yield 51 %.

4-(2-((5-Dimethylaminomethyl-2-furyl)methylthio)ethylamino)-3-methylfuroxan **(3a)**

To a stirred and ice water cooled solution of Na 0.10 g (4.3 mmol) in 10 ml of ethanol, 0.44 g (2.1 mmol) **4** was added slowly, under N_2. Then to the solution 0.30 g (2.1 mmol) **6** dissolved in ethanol (5 ml) was added. The stirred mixture was kept under N_2 for 1 h at room temp., filtered to remove the insoluble and evaporated under vacuum. The residue was purified by flash chromatography (eluents: first petroleum ether 40–60°: THF, 90:10 and afterwards methylene chloride:methanol, 90:10). The oil thus obtained (yield 72 %) was immediately transformed into the corresponding oxalate.

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