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Original article

Synthesis and pharmacological evaluation of 4,4a-dihydro-5H-[1]benzopyrano[4,3-*c*]pyridazin-3(2H)-ones bioisosters of antihypertensive and antithrombotic benzo[*h*]cinnolinones

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Summary — A series of 4,4a-dihydro-5H-[1]benzopyrano[4,3-*c*]pyridazin-3-(2H)-ones (**2a–h**), have been prepared and evaluated for their pharmacological profile as antihypertensive and antithrombotic agents. Compounds **2** were ineffective in lowering the blood pressure of spontaneously hypertensive rats (SHR), only **2c** (R_1 = $NHCOCH_3$) showing a short lasting action (< 2 h). Compounds **2c** and **2b** (R_1 = NH_2) were found to be very active as antithrombotic agents in mice, being more potent than acetylsalicylic acid (ASA) taken as reference drug. Moreover, many derivatives of this class protected rats from formation of ASA or phenylbutazone (PBZ) induced ulcers, the most active being **2f** (R_2 = OCH_3) (ED_{50} = 12.2 mg/kg and 25.4 mg/kg *po* in ASA and PBZ models, respectively).

Résumé — Synthèse et évaluation pharmacologique de 4,4a-dihydro-5H-[1]benzopyrano[4,3-*c*]pyridazin-3(2H)-ones bioisosters de benzo [h] **cinnolinones antihypertensives et antithrombotiques.** *Une série de 4,4a-dihydro-5H-[1]-benzopyran-[4,3-c]pyridazin-3(2H)-ones (**2a—h**) a été synthétisée et évaluée quant à son profil pharmacologique comme agents antihypertensifs et antithrombotiques. Les composés **2** sont dépourvus d'activité antihypertensive; seul **2c** (R_1 = $NHCOCH_3$) montre une action peu durable (< 2 h). Les produits **2c** et **2b** (R_1 = NH_2) manifestent une puissante activité antithrombotique chez la souris, et s'avèrent plus actifs que l'acide acétylsalicylique (ASA), pris comme référence. Plusieurs composés de cette série protègent les rats des ulcères provoqués par l'ASA ou la phénylbutazone (PBZ), le produit le plus actif étant **2f** (R_2 = OCH_3) (ED_{50} = 12,2 mg/kg et 25,4 mg/kg* po *dans les modèles du ASA et du PBZ, respectivement).*

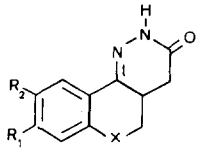
antihypertensive activity / antithrombotic activity / antiulcer activity / dihydro-5H-[1]benzopyrano[4,3-*c*]pyridazin-3-ones

Introduction

In a previous paper [1] we reported the synthesis and pharmacological profile of a series of 4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2H)ones **1**, among which **1b**, **c** were found to be potent antihypertensive and anti-thrombotic agents. These results induced us to extend our studies to the isosteric structure of 4,4a-dihydro-5H-[1]benzopyrano[4,3-*c*]pyridazin-3(2H)ones **2**.

Since some derivatives in the benzocinnolinone series showed antiulcer properties, which were however not dose-dependent, and since moreover the pyridazinonic moiety has recently been reported to possess antisecretory and antiulcer activities, [2] we focused our attention on more extensive studies, mostly directed towards discovering new compounds useful for gastric protection.

We report in this paper the synthesis and pharmacological evaluation of representative **2** in which the aryl moiety is either unsubstituted **2a** or substituted with an amino **2b** or an acetylamino group **2c**, **2d**. Substitution with methoxy group(s) **2e**, **f**, **h** or chloro **2g** is also considered.



1, X = CH_2

2, X = O

R_1R_2=H (a); R_1=NH_2 (b); R_1=$NHCOCH_3$ (c); R_2=$NHCOCH_3$ (d); R_1=OCH_3 (e); R_2=OCH_3 (f); R_2=Cl (g); R_1R_2=OCH_3 (h)

R = H when not expressly indicated

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Chemistry

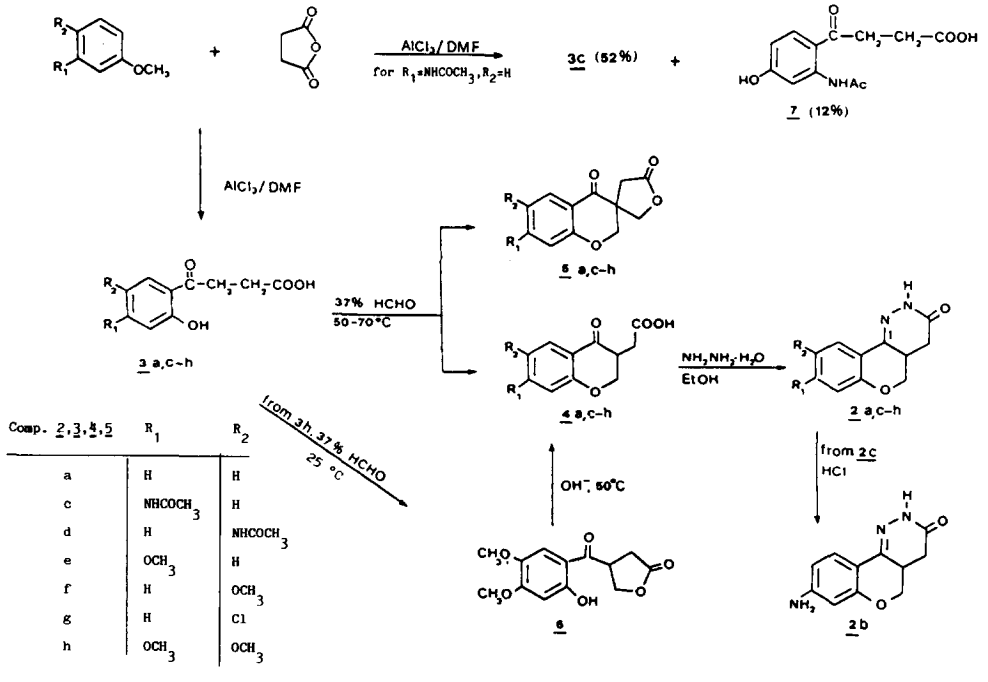
Target compounds were prepared by the route shown in scheme 1. Acylation of the appropriate benzene derivative with succinic anhydride and $AlCl_3$ under various conditions gave the known 2'-hydroxy-3-substituted benzoyl-propionic acids **3a, d–h** [3–8] and **3c**. It is to be noted that starting from *m*-acetylaminoanisole, the desired 2'-hydroxy-4'-acetamido derivative **3c** was accompanied by the 2'-acetamido-4'-hydroxy isomer **7** (see scheme 1). Separation of **3c** + **7** was achieved by crystallization from ethanol to give **3c**, followed by silica gel chromatography of the more soluble fraction enriched in **7**. The configurations of **3c** and **7** were attributed on the basis of their amidic NH IR absorption, which appeared as a clean sharp band at 3320 cm^{-1} for **3c** and as a broad signal near 3100 cm^{-1} for **7**, due to the hydrogen-bond between the amidic and the carbonyl functions. Hydroxymethylation of **3** with 37% formaldehyde and 0.5 N sodium hydroxide in a 1/1.1/2.1 ratio (v/v/v) at 50–70°C gave the 4-chromanon-3-acetic acids **4** beside traces of the spiroderivative **5**, with the exception of **5h** (R_1,R_2 = OCH_3) which was the main reaction product. A better procedure to **4h** was devised through the lactone **6** (see scheme 1). Final cyclization of **4** with hydrazine hydrate in refluxing ethanol led to the desired 4,4a-dihydro-5H-[1]-benzopyrano[4,3-*c*] pyridazin-3(2H)-ones **2**, whose structure was supported by analytical and spectral data. The amino derivative **2b** was obtained from the corresponding acetamido **2c** by refluxing in 2 N hydrochloric acid.

Finally, 8-acetylamino **1c** [1] and 9-methoxy-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2H)one **1f** were synthesized from the corresponding 1,2,3,4-tetrahydro-1-oxo-naphthalene-2-acetic acids according to a previously reported method [9].

Pharmacology

Compounds **2a–h** were submitted for evaluation of their antihypertensive, antithrombotic and antiulcer effects. Moreover, the antiulcer properties of benzo[*h*]cinnolinones **1c**, **f** have been tested in comparison with those of **2c**, **f**. For all derivatives, acute toxicity (mouse) was calculated.

The antihypertensive activity is reported as the variation of blood pressure of conscious spontaneously hypertensive rats (peak effect) at a single oral dose of 6.25 mg/kg, using dihydralizine as the reference drug. The antithrombotic activity *in vivo* was evaluated in the mouse by inducing death or paralysis of the hind limbs with a thrombotic mixture (fetal bovine collagen 200 µg/ml, adrenaline 200 µM). Values are reported as percent protection *vs* controls at doses equimolar to 20 mg/kg of acetylsalicyclic acid (ASA). The antiulcer activity was evaluated in



Scheme 1. Synthesis of compounds **2–7**.

the rat by the ASA- or phenylbutazone (PBZ)-induced ulcer models. It is reported in terms of doses that reduced the number and severity of gastric lesions by 50%, using *N*-[2-[[[-5-(dimethylamino)methyl-2-furanyl]-methyl]thio]ethyl]-*N'*-methyl-2-nitro-1,1-ethene-diamine (ranitidine) as the reference drug.

Table I. Antihypertensive and antithrombotic properties of compounds **2b, c.**

Compd	R_1	R_2	*Antihypertensive* [a] *activity (SHR) ΔBP (mmHg) ± SD*	*Antithrombotic* [b] *activity (mouse)*
2b	NH_2	H	−16 ± 5.16	84*
2c	$NHCOCH_3$	H	−40 ± 7.91	75*
ASA				39*
Dihydralazine			−75 ± 6.15	

[a]Peak effect at an oral dose of 6.25 mg/kg. Results are means for 6 animals. [b]Protection *vs* controls. Dose equimolar to 20 mg/kg ASA. Groups of 10 animals. Statistical comparison with χ^2 test for mortality in treated animals *vs* controls (*$P < 0.01$).

Results and Discussion

Among the test compounds the amino **2b** and the acetylamino derivative **2c** showed antithrombotic effects higher than those of ASA in mice, while **2a**, **d–h** were devoid of antithrombotic properties.

None of them possessed antihypertensive activity, only **2c** displaying a significant but short lasting (< 2 h), reduction of blood pressure in spontaneously hypertensive rats (SHR) (table I).

All compounds have been tested for their activity in protecting rats against ASA- and PBZ-induced gastric lesions. When substantial activity (> 50%) was observed at a dose of 100 mg/kg, full dose range studies were performed and the ED_{50} values were determined as shown in table II.

A close inspection of the results reveals some interesting facts with respect to structure–activity relationships. The unsubstituted derivative **2a** was effective in both ASA and PBZ models, though it was 20-fold less active than the reference drug. Introduction of an acetylamino group at the C_8 **2c** led to a more active compound in the ASA model. However, it was inactive in the PBZ model. Shifting the acetylamino group of **2c** to the C_9 **2d** completely suppressed the activity. On the contrary, the 9-methoxy derivative **2f** had the most potent antiulcer activity in this series.

Table II. Antiulcer activity of compounds **1c**, **f** and **2a–c**, **f**, **h**.

Compd	R_1	R_2	LD_{50} (po) *mg/kg (mouse)*	*Inhibition of rat gastric ulcers*[a] ED_{50}, *mg/kg*, po	
				ASA	*PBZ*
2a	H	H	1 000	24.8 22.4–27.2	58.1 50.9–65.3
2b	NH_2	H	536	[b]	NA[c]
2c	$NHCOCH_3$	H	1 000	10.0 6.4–13.7	NA
2f	H	OCH_3	500	12.2 10.8–13.6	25.4 24.0–26.7
2h	OCH_3	OCH_3	1 000	53.5 49.2–57.8	36.9 33.0–40.8
1c	$NHCOCH_3$	H	1 000	9.9 7.4–11.4	NA
1f	H	OCH_3	1 000	10.3 9.2–11.4	100
Ranitidine			884	1.2 1.1–1.3	2.9 2.6–3.1

[a]Rat gastric ulcers induced by ASA (100 mg/kg *po*) or PBZ (200 mg/kg *po*) and confidence limits for $P = 0.05$. [b]Compound displays a non dose-dependent activity. [c]Not active up to 100 mg/kg.

Moving the methoxy group to the 8 position **2e** as well as replacing it with a chloro atom **2g** determined loss of activity. Introduction of an additional methoxy group at position 8 of **2f** (compound **2h**) reduced the antiulcer properties, notably in the ASA model.

Finally, a comparison has been made with 2 bioisoster benzocinnolinone derivatives (**2c** *vs* **1c** and **2f** *vs* **1f**). It was noteworthy that, while in one case (**2c** *vs* **1c**) both compounds displayed the same properties, being active in the ASA model and inactive in the PBZ model, the second pair displayed quite different behaviour. In fact, **2f** was found to be much more potent than **1f** in the PBZ model, while their activity was still comparable in the ASA model.

The most interesting compound, **2f**, has been selected for a more complete pharmacological study. Preliminary results are shown in table III.

As can be seen from the data, *in vitro* experiments revealed that it has no anticholinergic activity at 1 x 10^{-5} M and no histamine H_2-receptor antagonistic activity at the same concentration. On the contrary, it has a potent antisecretory activity, and in addition exhibits gastroprotective activity in the rat against ethanol-induced lesions. A hypothesis could be made that **2f** exerts antiulcer activity by stimulating endogenous prostaglandins production. However, its activity is not lowered in rats pretreated with indomethacin (data not reported), thus suggesting that a different mechanism may be involved.

A further pharmacological and toxicological investigation of this compound is currently in progress to establish if it is representative of a novel class of agents displaying gastric antiulcer and antisecretory activity, being neither a competitive histamine (H_2) or acetylcholine receptor antagonist nor prostaglandin analog.

Experimental Protocols

Chemistry

Melting points were determined with a Büchi 510 capillary apparatus and are uncorrected. Analyses indicated by the symbols of the elements or functions were within ± 0.4 of the theoretical values. IR spectra (nujol mull, unless otherwise noted) were recorded on a Perkin–Elmer 1310 infrared spectrophotometer. The following abbreviations are used to define the band characteristics: s (strong), b (broad), m (medium), w (weak). ^{1}H NMR spectra were recorded in DMSO–d_6 (unless otherwise noted) on a Hitachi Perkin–Elmer R 600 FT spectrometer, with tetramethylsilane as the internal standard.

*2'-Hydroxy-3-benzoyl-propionic acid **3a***

Compound **3a** was prepared according to the method of LF Fieser *et al* [3]. mp: 140–41°C (lit 139–40°C) [3]. IR: 1710 cm^{-1} (m, acid C=O); 1690 cm^{-1} (s, ketone C=O); ^{1}H NMR ($CDCl_3$) δ: 2.8 (t, J = 6 Hz, 2H, CH_2); 3.4 (t, J = 6 Hz, 2H, CH_2); 6.7–8.1 (m, $4H_{arom}$ + COOH); 12.2 (s, 1H, 0H; exchanges with D_2O). Anal $C_{10}H_{10}O_4$ (C, H).

*2'-Hydroxy-4'-acetamido-3-benzoyl-propionic acid **3c** and 2'-acetamido-4'-hydroxy 3-benzoyl-propionic acid **7***

DMF (6.6 ml) was cautiously added to anhydrous $AlCl_3$ (40 g, 0.37 mol) under stirring, followed by rapid addition of a finely ground mixture of *m*-acetamidoanisole (5 g, 0.03 mol) and succinic anhydride (3.5 g, 0.035 mol). After heating at 70°C for 1 h, the mixture was cooled, poured onto ice and acidified with 4 N HCl. The precipitate that was formed was filtered, washed with water and refluxed 15 min with 5% $NaHCO_3$. The insoluble was filtered off and the cooled solution acidified to give 5.6 g of a precipitate formed by a mixture of **3c** and **7**. This solid was refluxed with ethanol (50 ml) and the insoluble filtered still hot to yield 3.5 g (46%) of pure **3c**. The mother liquors were evaporated to dryness and the residue was purified by chromatography (Merck silica gel 60, 70–230 mesh; eluent $CHCl_3$/MeOH, 9/1) to yield in the order additional 0.4 g (total yield 3.9 g, 52%) of **3c** and 0.8 g (12%) of **7**.

For **3c** mp = 252–54°C. IR = 3320 cm^{-1} (s, NH); 1690 cm^{-1} (m, acid C=O); 1630 cm^{-1} (s, ketone C=O + amide C=O). ^{1}H NMR δ: 2.1 (s, 3H, CH_3); 2.6 (t, J = 6 Hz, 2H, CH_2); 3.3 (t, J = 6 Hz, 2H, CH_2); 6.9–8.2 (m, $3H_{arom}$); 10.21 (s, 1H, NH; exchanges with D_2O); 12.01 (s, 1H, OH; exchanges with D_2O). Anal $C_{12}H_{13}NO_5$ (C, H, N).

For **7**: mp = 250°C. IR = 3200–3000 cm^{-1} (b, NH); 1690 cm^{-1} (m, acid, C=O); 1660 cm^{-1} (m, ketone C=O); 1620 cm^{-1} (m, amide C=O). ^{1}H NMR δ: 2.1 (s, 3H, CH_3); 2.5 (t, J = 6 Hz, 2H, CH_2); 3.4 (t, J = 6 Hz, 2H, CH_2); 6.7–8.0 (m, $3H_{arom}$); 12.1 (s, 1H, OH; exchanges with D_2O). Anal $C_{12}H_{13}NO_5$ (C, H, N).

Table III. Biological properties of compound **2f**.

Compd	*Inhibition of EtOH ulcers*[a] *(rat)*	*Antisecretory activity*[b] *gastric acid output (rat)*	*Anti H_2 activity*[c] *(guinea pig)*	*Anticholinergic activity*[d] *(guinea pig)*
2f	41	69	NA[e]	NA
Ratinidine	NA	63	$pA_2 = 7.50 \pm 0.05$[f]	–
Pirenzepine	NA	87	–	$pA_2 = 6.28 \pm 0.07$

[a]Percent inhibition *vs* controls at a dose of 30 mg/kg *po*. [b]Percent inhibition *vs* controls at a dose of 30 mg/kg ip. [c]Tested *in vitro* on guinea pig atrium preparation up to 1 x 10^{-5} M. [d]Tested *in vitro* on guinea pig ileum preparation up to 1 x 10^{-5} M. [e]Not active. [f]Mean ± SD calculated from dose–response curves, according to Van Rossum [10].

*2'-Hydroxy-5'-acetamido-3-benzoyl-propionic acid **3d***
Compound **3d** was prepared starting from *p*-acetamidoanisole according to the method above reported for **3c**. Yield 93%; mp = 181–85°C [4]. IR = 3500 cm^{-1} (m, phenol OH); 3300 cm^{-1} (m, amide NH); 1720 cm^{-1} (s, acid C=O); 1690 cm^{-1} (s, ketone C=O); 1620 cm^{-1} (s, amide C=O). ^1H NMR δ: 2.05 (s, 3H, CH_3); 2.6 (t, J = 6 Hz, 2H, CH_2); 3.3 (t, J = 6 Hz, 2H, CH_2); 7.2–8.0 (m, $3H_{arom}$); 10.1 (s, 1H, NH; exchanges with D_2O); 12.0 (s, 1H, OH; exchanges with D_2O). Anal $C_{12}H_{13}NO_5$ (C, H, N).

*2'-Hydroxy-4'-methoxy-3-benzoyl-propionic acid **3e***
A mixture of 1,3-dimethoxybenzene (15 g, 0.1 mol) and succinic anhydride (10 g, 0.1 mol) was added portionwise to $AlCl_3$ (10 g, 0.075 mol) and heated at 100°C for 3 h under stirring. After cooling, the reaction mixture was poured onto ice and acidified with 2 N HCl. The resulting precipitate was filtered and crystallized from water to give 15.7 g (70%) of **3e**. mp = 155–56°C [5]. IR = 3400 cm^{-1} (b, phenol OH); 1700 cm^{-1} (s, acid C=O); 1640 cm^{-1} (s, ketone C=O). ^1H NMR δ: 2.8 (t, J = 6 Hz, 2H, CH_2); 3.36 (t, J = 6 Hz, 2H, CH_2); 3.9 (s, 3H, OCH_3); 7.2–8.0 (m, $3H_{arom}$); 11.4 (s, 1H, OH; exchanges with D_2O). Anal $C_{11}H_{12}O_5$ (C, H).

*2'-Hydroxy-5'-methoxy-3-benzoyl-propionic acid **3f***
To a mechanically stirred mixture of 1,4-dimethoxybenzene (32 g, 0.23 mol) and succinic anhydride (24 g, 0.24 mol) in nitrobenzene (200 ml) at 10°C, $AlCl_3$ (64 g, 0.48 mol) was added portionwise. At the end of the addition the mixture was stirred at 60°C for 3 h, cooled, poured onto ice and acidified with 6 N HCl. The solvent was removed with steam and the residue was taken up in warm dilute Na_2CO_3. After being filtered from alumina, the solution was treated with Norit, cooled in ice and acidified with 6 N HCl. The precipitate was then crystallized from ethanol to give 33.5 g (65%) of **3f**. mp = 137–40°C (lit 137–42°C) [6]. IR = 1700 cm^{-1} (m, acid C=O); 1680 cm^{-1} (s, ketone C=O). ^1H NMR δ: 2.72 (t, J = 6 Hz, 2H, CH_2); 3.32 (t, J = 6 Hz, 2H, CH_2); 3.8 (s, 3H, OCH_3); 6.7–7.4 (m, $3H_{arom}$); 10.4 (s, 1H, OH). Anal $C_{11}H_{12}O_5$ (C, H).

*2'-Hydroxy-5'-chloro-3-benzoyl-propionic acid **3g***
Compound was prepared according to the method reported for **3f**, starting from 4-chlorophenol. Yield 88%. mp = 173–74°C (lit 180–81°C) [7]. IR = 1690 cm^{-1} (m, acid C=O); 1650 cm^{-1} (s, ketone C=O). ^1H NMR δ: 2.6 (t, J = 7 Hz, 2H, CH_2); 3.36 (t, J = 7 Hz, 2H, CH_2); 6.8–8.0 (m, $3H_{arom}$); 11.6 (s, 1H, OH). Anal $C_{10}H_9ClO_4$ (C, H, Cl).

*2'-Hydroxy-4',5'-dimethoxy-3-benzoyl-propionic acid **3h***
Compound was prepared according to Murata *et al* [8] mp = 152–55°C (lit 162–63°C). IR = 3300 cm^{-1} (b, OH); 1700 cm^{-1} (m, acid C=O); 1650 cm^{-1} (s, ketone C=O). ^1H NMR δ: 2.8 (t, J = 6 Hz, 2H, CH_2); 3.3 (t, J = 6 Hz, 2H, CH_2); 3.9 (app d, 6H); 6.4 (s, $1H_{arom}$); 7.0 (s, $1H_{arom}$); 9.8 (s, 1H, OH). Anal $C_{12}H_{14}O_6$ (C, H).

*4-Chromanon-3-acetic acids **4** and spiroderivatives **5**. General method*
A solution of the appropriate 2'-hydroxy-3-benzoyl-propionic acid **3** (1 mol) and 37% HCHO (1.1 mol) in 0.5 NaOH (2.1 mol) was heated at 70°C for 1 h. When starting **3** is acetamido substituted, the reaction temperature did not exceed 50°C, in order to avoid hydrolysis of the amide function. After cooling, acidification (pH 4) of the mixture led to the precipitation of a first crop of the desired **4**. Additional **4**, contaminated by the spiroderivative **5**, could be isolated from the aqueous filtrate by exraction in continuous with 300 ml of chloroform, followed by silica gel chromatography of the extract (Merck silica gel, 60, 70–230 mesh; eluent $CHCl_3$/MeOH 9/1 v/v). The spiroderivative **5** was collected as the forerun (see table IV).

In the case of **3h**, however, an unsatisfactory yield of **4h** (<10%) was obtained, **5h** being the main reaction product (~ 30%). An increased ratio of formaldehyde (HCHO/**3** = 2/1) led to the isolationof **5** in yield of up to 65%.

*3,4-Dihydro-6,7-dimethoxy-4-oxo-2H-1-benzopyrane-3-acetic acid **4h***
A mixture of **3h** (6.6 g, 0.026 mol), 37% HCHO (2.12 ml, 0.028 mol) and 0.5 N NaOH (104 ml, 0.052 mol) was stirred at room temperature for 8 h. After acidification with 6 N HCl to pH 4, the mixture was stirred for 12 h. After extraction with chloroform (3 x 100 ml) the residue was chromatographed on silica gel (Merck silica gel, 60, 70–230 mesh; eluent chloroform) to give 3 g (43%) of 4-(2-hydroxy-4,5-dimethoxybenzoyl)-2(3H)-4,5-dihydrofuranone (**6**). mp = 135–36°C. IR = 1770, 1788 cm^{-1} (s, C=O). ^1H NMR δ: 2.6–3.1 (m, 2H, CH_2); 3.8 (s, 3H, OCH_3); 3.9 (s, 3H, OCH_3); 4.2–4.7 (m, 3H, CH–CH_2); 6.4 (s, $1H_{arom}$); 6.8 (s, $1H_{arom}$); 9.3 (s, 1H, OH; exchanges with D_2O). Anal $C_{13}H_{14}O_6$ (C, H).

A mixture of lactone **6** (1 g, 0.0038 mol) and 0.5 N NaOH (8 ml, 0.004 mol) was stirred for 1 h at 50°C. After cooling, the resulting solution was acidified, stirred for 2 h at room temperature and extracted with chloroform. After evaporation of the solvent the residue was chromatographed (Merck silica gel, 60, 70–230 mesh; $CHCl_3$/MeOH 9/1) to give 0.4 g (40%) of **4h** (see table IV).

*4,4a-Dihydro-5H[1]benzopyrano[4.3-c]pyridazin-3(2H)-ones **2**. General method*
A mixture of the required **4** (1 mol) and hydrazine hydrate (2 mol) in ethanol (1 litre) was refluxed for 3 h. After cooling, the insoluble **2** was filtered. Additional **2** could be isolated from the filtrate by evaporation of the solvent and purification of the residue by silica gel chromatography (Merck silica gel, 60, 70–230 mesh; $CHCl_3$/MeOH 9/1) (see table V).

*8-Amino-4,4a-dihydro-5H[1]benzopyrano[4,3-c]pyridazin-3(2H)-one **2b***
A suspension of **2c** (2.5 g, 0.0097 mol) in 2 N HCl (50 ml) was refluxed for 1 h. After cooling, the solution was brought to pH 5 with 5% $NaHCO_3$ and the precipitate which formed was filtered and washed with water. Yield 72%. mp = 242–45°C. IR = 3460, 3330, 3220 cm^{-1} (b, NH, NH); 1660 cm^{-1} (m, amide C=O). ^1H NMR δ: 2.0–2.6 (m, 2H, CH_2); 3.0–4.5 (m, 3H, CH–CH_2); 5.3 (br, s, 2H, NH_2; exchanges with D_2O); 6.0–6.4 (m, $3H_{arom}$). Anal $C_{11}H_{11}N_3O_2$ (C, H, N).

*9-Methoxy-4,4a,5,6-tetrahydrobenzo[h]cinnolin-3(2H)-one **1f***
Compound **1f** was prepared according to a previously reported method [9], starting from 7-methoxy-1,2,3,4-tetrahydro-1-oxo-naphthalene-2-acetic acid. Yield 50%. mp = 161–63°C. IR = 3100 cm^{-1} (m, NH); 1670 cm^{-1} (m, C=O). ^1H NMR δ: 1.8–3.0 (m, 7H); 3.8 (s, 3H, OCH_3); 6.7–7.8 (m, $3H_{arom}$); 8.8 (br s, 1H, NH; exchanges with D_2O). Anal $C_{13}H_{14}N_2O_2$ (C, H, N).

Pharmacology

Antihypertensive activity
Experiments were performed on unanesthetized rats (Charles River) weighing 150–200 g. Rats, 12-h fasted, were warmed at 33°C in a heating chamber for 30 min prior to blood pressure

determination. Groups of 6 animals/dose were employed. Systolic blood pressure was measured by the tail-cuff method, utilizing a tail plethismographic apparatus W + W BP recorder 8002. Test compounds were suspended in 1% methylcellulose and administered in a volume of 10 ml/kg by oral gavage at a dose of 6.25 mg/kg. Systolic blood pressure was recorded every hour for 6 h after drug administration. Dihydralazine was used as standard drug.

Antithrombotic activity in vivo

The determination was carried out by a modification of the method of Di Minno and Silver [11]. Male Swiss mice (Charles River) weighing 20–30 g were divided into 3 groups of 10. Groups 1 and 2 were treated with test compounds and ASA as a reference drug, respectively, both dissolved and diluted in 1% methylcellulose and administered ip in a vol of 10 ml/kg. The dose of the test compound was equimolar to that of ASA (20 mg/

Table IV. Physical properties of compounds **4** and **5**.

Compd	*Yield (%)*[a]	*mp (°C) solvent*	*Molecular formula*	*^1H NMR δ (ppm)*
4a	42	140–08 (ethanol)	$C_{11}H_{10}O_4$	2.5–2.8 (m, 2H, CH_2); 3.8–4.0 (m, 3H, CH-CH_2); 6.9–8.1 (m, $4H_{arom}$).
4c	45	250 (acetic ac)	$C_{13}H_{13}NO_5$	2. 1 (s, 3H, CH_3): 3.0–3.5 (m, 2H, CH_2); 3.8–4.2 (m, 3H, CH-CH_2); 6.0–6.5 (m, $2H_{arom}$); 7.3–7.9 (m, $1H_{arom}$).
4d	35	250 (dec) (acetic ac)	$C_{13}H_{13}NO_5$	2.1 (s, 3H, CH_3); 3.1–3.6 (m, 2H, CH_2); 4.1–4.7 (m, 3H, CH-CH_2); 7.1 (m, $1H_{arom}$); 7.7–8.3 (m, $2H_{arom}$).
4e	45	136–38 (ethanol)	$C_{12}H_{12}O_5$	2.9–3.3 (m, 2H, CH_2); 3.9 (s, 3H, OCH_3); 4.0–4.6 (m, 3H, CH-CH_2); 7.3–8.1 (m, $3H_{arom}$).
4f	48	173–75 (ethanol)	$C_{12}H_{12}O_5$	3.0–3.6 (m, 2H, CH_2); 3.7 (s, 3H, OCH_3); 4.0–4.6 (m, 3H, CH-CH_2); 6.8–7.3 (m, $3H_{arom}$).
4g	28	148–50 (ethanol)	$C_{11}H_9ClO_4$	2.1–2.8 (m, 2H, CH_2); 3.0–3.5 (m, 1H, CH); 3.8 (s, 3H, OCH_3); 4.0–4.7 (m, 2H, CH_2); 6.7–7.9 (m, $3H_{arom}$).
4h	40[b]	167–70 (ethanol)	$C_{13}H_{14}O_6$	2.2–2.9 (m, 2H, CH_2); 3.0–3.4 (m, 1H, CH); 3.7 (s, 3H, OCH_3); 3.8 (s, 3H, OCH_3); 4.0–4.6 (m, 2H, CH_2); 6.3 (s, $1H_{arom}$); 7.1 (s, $1H_{arom}$).
5a	10	107–08	$C_{12}H_{10}O_4$	2.5–3.2 (dd, J = 10 Hz, 2H, CH_2); 4.5 (s, 4H, $2CH_2$); 7.0–8.0 (m, $5H_{arom}$).
5d	20	195–96	$C_{14}H_{13}NO_5$	2.1 (s, 3H, CH_3); 2.7 (s, 2H, CH_2); 4.4 (s, 4H, $2CH_2$); 7.0–7.9 (m, $3H_{arom}$).
5f	9	145–46	$C_{13}H_{12}O_5$	2.5–3.2 (dd, J = 10 Hz, 2H, CH_2); 3.9 (s, 3H, OCH_3); 4.4 (s, 4H, $2CH_2$); 6.8–7.3 (m, $3H_{arom}$).
5g	8	117–18	$C_{12}H_9ClO_4$	2.3–3.4 (dd, J = 10 Hz, 2H, $2CH_2$); 4.4 (s, 4H, $2CH_2$); 6.9–7.9 (m, $3H_{arom}$).
5h	27	178–79	$C_{14}H_{14}O_6$	2.2–3.3 (dd, J = 10 Hz, 2H, CH_2); 3.8 (s, 3H, OCH_3); 3.9 (s, 3H, OCH_3); 4.3 (s, 4H, $2CH_2$); 6.3 (s, $1H_{arom}$); 7.1 (s, $1H_{arom}$).

[a]Molar ratio **3**/HCHO/NaOH 1/1.1/2.1. [b]See *Experimental protocols* for synthesis.

kg). One h after medication groups 1 and 2, along with group 3 (controls), received a thrombotic mixture (fetal bovine collagen 200 μg/ml, prepared by Stago Laboratories, Sannois (France) and epinephrine 200 μM (Stago Lab) in Michaelis buffer (pH 7.6, sodium barbital (5.33 g), sodium acetate (2.12 g), sodium chloride (6.31 g), hydrochloric acid (1.40 ml), water (1 000 ml), in a vol of 10 ml/kg, administered iv in the tail at a speed of 20 μg/sec (before injection animals were warmed at 27°C for 30 min).

Death of animals or paralysis for more than 15 min of the hind limbs were considered as thrombotic effects. The antithrombotic activity of the test compound was measured as % protection (% P) by relating the number of the thrombotic effects in group 1 (N_t, treated) to those of group 3 (N_c, controls), according to the formula $\% P = \frac{N_c - N_t}{N_c} \cdot 100$. In the same way the protection of ASA was calculated comparing groups 2 and 3.

Antiulcer activity

Male Sprague–Dawley rats (Charles River) weighing about 145–155 g, in groups of 8, were housed in individual cages and fasted for 24 h with free access to water. Test compounds were administered by gavage 1 h before acetylsalicylic acid (100 mg/kg *per os*), phenylbutazone (200 mg/kg *po*) or 95% EtOH (1 ml *po*), while the control group received distilled water only. Four h later, animals were sacrified and their stomachs excised and opened along the greater curvature. The number and severity of lesions was observed with a 10 x wide field binocular microscope and evaluated with the method proposed by Moron *et al* [12]. The ED_{50} values for quantitative data were calculated by linear regression analysis, with confidence limits for $P = 0.05$.

Acute toxicity

LD_{50} was determined in male Swiss mice (Charles River), weighing 20–30 g, according to the Litchfield and Wilcoxon method [13].

Gastric antisecretory activity

Gastric antisecretory activity was evaluated using the technique of Shay *et al* [14]. Male Sprague–Dawley rats (Charles River) weighing 150–200 g, were fasted for 48 h prior to the test; during the fasting period animals were treated 3 times with 5% fructose in distilled water (2 mg/100 g). After fasting, the rats were divided into groups of 6 animals each. One group served as the control. A small midline incision was performed and the pylorus was ligated under ether anesthesia. The test compound suspended in 1% methylcellulose was administered intraperitoneally at a dose of 30 mg/kg. Four h after drug administration, the animals were killed and the stomachs removed. The contents of the stomachs were collected and the volume was recorded. The gastric juice was titrated against 0.1 N NaOH to determine the acid output at a pH end-point of 7.0. Percent inhibition *vs* controls was calculated.

Anticholinergic activity

The anticholinergic activity was determined using the guinea

Table V. Physical properties of compounds **2.**

Compd	*Yield (%)*	*mp (°C)*	*Molecular formula*	*^1H NMR δ (ppm)*
2a	79	212–14	$C_{11}H_{10}N_2O_2$	2.2–2.7 (m; 2H, CH_2); 3.2–4.8 (m, 3H, CH-CH_2); 7.0–8.0 (m, $4H_{arom}$).
2c	85	250	$C_{13}H_{13}N_3O_3$	2.1 (s, 3H, CH_3); 2.2–2.8 (m, 2H, CH_2 3.0–4.8 (m, 3H, CH-CH_2); 6.9–8.1 (m, $3H_{arom}$).
2d	75	250	$C_{13}H_{13}N_3O_3$	2.1–2.7 (m, 5H, CH_2+CH_3); 3.5–4.7 (m, 3H, CH-CH_2); 6.9 (m, $1H_{arom}$); 7.3–7.7 (m, $1H_{arom}$); 8.2 (m, $1H_{arom}$).
2e	68	205–10	$C_{12}H_{12}N_2O_3$	2.2–2.7 (m, 2H, CH_2); 3.7 (s, 3H, OCH_3); 3.8–4.5 (m, 3H, CH-CH_2); 6.5–7.8 (m, $3H_{arom}$).
2f	84	219–20	$C_{12}H_{12}N_2O_3$	2.2–2.8 (m, 2H, CH_2); 3.7 (s, 3H, OCH_3); 3.9–4.6 (m, 3H, CH-CH_2); 6.9–7.5 (m, $3H_{arom}$).
2g	84	250	$C_{11}H_9ClN_2O_2$	2.1–2.5 (m, 2H, CH_2); 3.0–4.5 (m, 3H, CH-CH_2); 6.7–7.8 (m, $3H_{arom}$).
2h	90	231–32	$C_{13}H_{14}N_2O_4$	2.1–2.5 (m, 2H, CH_2); 3.0–4.4 (m, 3H, CH-CH_2); 3.7 (s, 6H, OCH_3); 6.3 (s, $1H_{arom}$); 7.1 (s, $1H_{arom}$).

pig isolated ileum preparation suspended, under a tension of 1 g, in Tyrode solution aerated with 95% 0_2 + 5% CO_2 at 37°C. Cumulative dose–response curves for acetylcholine-induced contractions were determined in the absence or in the presence of the test compound (1×10^{-6} to 1×10^{-5} M) or pirenzepine (3×10^{-7} to 1×10^{-6} M).

Histamine H_2-receptor antagonistic activity

The activity was determined using the guinea pig isolated right atrium preparation suspended in Krebs–Ringer solution aerated with 95% O_2 + 5% CO_2 at 32°C. Cumulative dose–response curves for histamine-induced positive chronotopic action were determined in the absence or in the presence of the test compound (1×10^{-6} to 1×10^{-5} M) or ranitidine (3×10^{-7} to 1×10^{-6} M).

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