Synthesis and Biological Evaluation of Substituted Benzo[*h*]cinnolinones and 3*H*-Benzo[6,7]cyclohepta[1,2-*c*]pyridazinones: Higher Homologues of the Antihypertensive and Antithrombotic 5*H*-Indeno[1,2-*c*]pyridazinones

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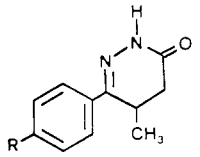
Several substituted benzo[*h*]cinnolinones **3** and 3*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazinones **4**, which were designed as less rigid congeners of 5*H*-indeno[1,2-*c*]pyridazinones **2**, were synthesized and tested as antihypertensive, inotropic, antithrombotic, antiinflammatory, and antiulcer agents. While the seven-membered ring derivatives displayed only antithrombotic properties, which were comparable to that of acetylsalicylic acid, most of the benzo[*h*]cinnolinones exhibited significant antihypertensive, inotropic, and antithrombotic properties. In this respect, the 8-amino (**3b**) and 8-acetylamino (**3c**) together with the 4,4a-dehydro analogue of **3c** (**11**) were found to possess the most potent and long-lasting antihypertensive activity. In particular, the dextro isomer of **3c** was more active than the racemic form, with lower tachycardiac effects. Unlike the lower homologues **2**, none of the compounds showed significant antiinflammatory or antiulcer activity.

In previous papers[1,2] we reported the synthesis and the pharmacological profile of 7-substituted-4,4a-dihydro-5*H*-indeno[1,2-*c*]pyridazinones (**2**) which are rigid congeners of the antihypertensive 6-aryl-5-methyl-4,5-dihydro-3-(2*H*)-pyridazinones (**1**).[3-5]



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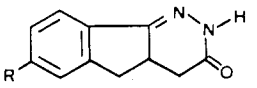
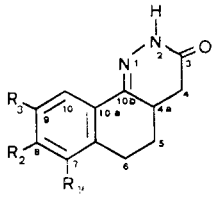
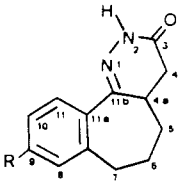


2 a-d



3 a-g



4 b-c

R = CN (a), NH_2 (b), $NHCOCH_3$ (c), $NHCOCH(Cl)CH_3$ (d); R_1 = $NHCOCH_3$ (e); R_2 = CN (a), NH_2 (b), $NHCOCH_3$ (c), $NHCOCH(Cl)CH_3$ (d); R_3 = $NHCOCH_3$ (f); R_1, R_2, R_3 = H when not expressly indicated.

Unlike 7-cyano derivatives **2a**, which displays only antiinflammatory action, the 7-amino (**2b**) and the 7-acetylamino (**2c**) derivatives were found to be potent antihypertensive and antithrombotic agents; in addition, **2c** as well as the 7-(2-chloropropionyl) derivative (**2d**) were highly effective in inhibiting indomethacin-induced ulcers in rats.[2]

The interesting and somewhat unexpected properties of **2b–d** induced us to extend our investigation to the related, less rigid structures **3** and **4**, in which the central hexa- or heptaatomic ring allows a mutual partial rotation of the aryl and dihydropyridazinone moieties. The purpose of this research was to ascertain whether the planarity of structure **2** is consistent with maximum activity.

Accordingly, we synthesized and tested a series of higher homologues of **2a–d** having the 4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2H)-one (**3a-g**) and the 2,4,4a,5,6,7-hexahydro-3*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazin-3-one (**4b,c**) structure. A recent paper[6] from another laboratory reporting the potent positive inotropic activity of **2c**, which decreases in the cinnoline homologue **3c**, has prompted us to publish our chemical and biological results.

Chemistry

The synthesis of 8-(acetylamino)-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3-one (**3c**) and the higher homologue 9-(acetylamino)-2,4,4a,5,6,7-hexahydro-3*H*-benzo[6,7]-cyclohepta[1,2-*c*]pyridazin-3-one (**4c**) was accomplished by following a similar procedure to that employed for **2c**.[2] As depicted in Scheme I, 6-(acetylamino)tetralone (**5c**)[7] was condensed with glyoxylic acid to give unsaturated acid **6c**, which was reduced with zinc in acetic acid to give 6-(acetylamino)-1,2,3,4-tetrahydro-1-oxo-1*H*-naphthalene-2-acetic acid (**7c**). The reaction of **7c** with hydrazine hydrate in refluxing ethanol led to **3c** in a 51% overall yield. This method markedly improved a reported alternative conversion of **5c** into **3c** occurring with a 22% overall yield.[6] Similarly, 7-(acetylamino)-1-benzosuberone (**8c**)[7] was converted into **4c** through the intermediates **9c** and **10c** (see Scheme I). Hydrolysis of **3c** and **4c**, in refluxing hydrochloric acid, respectively led to the amino derivatives **3b** and **4b**.

In order to prepare the enantiomers of the active analogue **3c**, racemic intermediate **7c** was treated with (+)-α-methylbenzylamine in ethanol. Crystallization of the less soluble fraction followed by treatment with hydrochloric acid led to (+)-**7c**. The levo-enriched form was recovered and purified from the mother liquor by treat-

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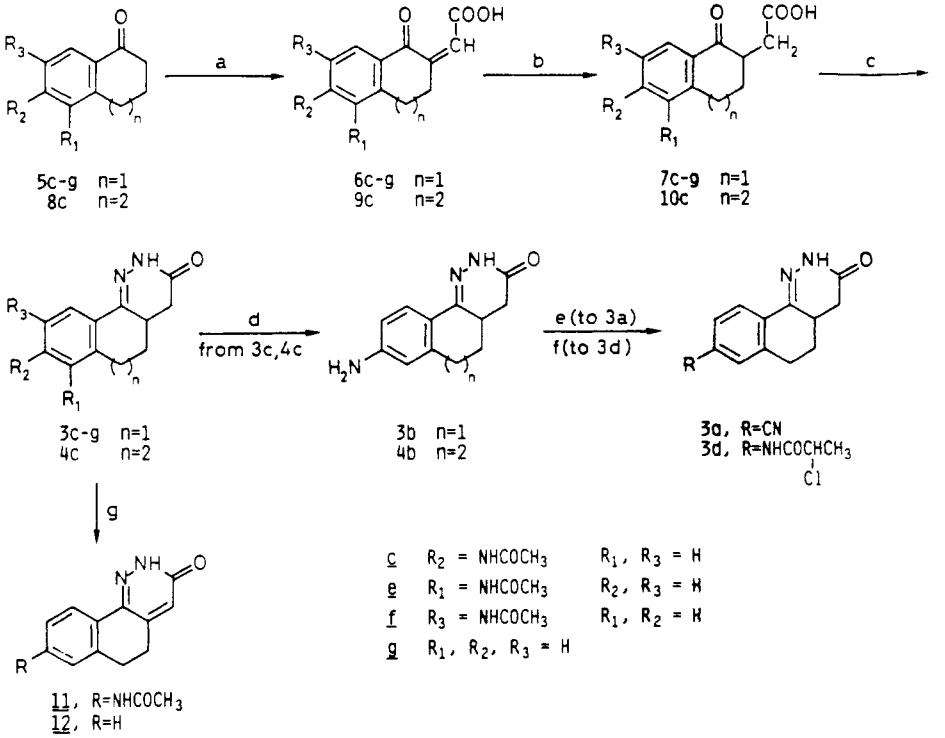
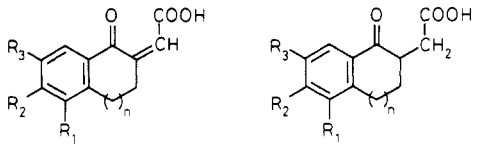
Scheme I[a]



[a] (a) Glyoxylic acid; (b) Zn, AcOH; (c) $NH_2NH_2 \cdot H_2O$, EtOH; (d) 4 N HCl; (e) $NaNO_2$, KCN; (f) MeCH(Cl)COCl; (g) sodium *m*-nitrobenzenesulfonate, NaOH.

Table I. Physical Properties for **6**, **7**, **9**, and **10**



compd	R_1	R_2	R_3	mp, °C	recrystn solvent	% yield	formula[a]
6e	$NHCOCH_3$	H	H	210–12 dec	ethanol	50	$C_{14}H_{13}NO_4$
6f	H	H	$NHCOCH_3$	222–24 dec	ethanol	70	$C_{14}H_{13}NO_4$
7e	$NHCOCH_3$	H	H	158–60	ethanol	60	$C_{14}H_{15}NO_4$
7f	H	H	$NHCOCH_3$	185–87	ethanol	68	$C_{14}H_{15}NO_4$
9c	H	$NHCOCH_3$	H	240–43	ethanol	85	$C_{15}H_{15}NO_4$
10c	H	$NHCOCH_3$	H	167–68	acetic acid	70	$C_{15}H_{17}NO_4$

[a] Analytical results for C, H, and N were within ±0.4% of theoretical values.

ment with (−)-α-methylbenzylamine. Final cyclization of (+)-**7c** and (−)-**7c** with hydrazine hydrate in ethanol afforded (−)-**3c** and (+)-**3c**, respectively.

8-Cyano derivative **3a** was prepared from **3b** by the Sandmeyer reaction in the presence of copper(I) cyanide. Acylation of **3b** with 2-chloropropionyl chloride in toluene led to **3d**. The 7-(acetylamino)- (**3e**) and the 9-(acetylamino)- (**3f**) benzocinnolinones were obtained by following the procedure described for **3c**, starting from the known 5-(acetylamino)tetralone[8] (**5e**) and 7-(acetylamino)tetralone[9] (**5f**), respectively. Oxidation of **3c** to 8-(acetylamino)-5,6-dihydrobenzo[*h*]cinnolin-3(2*H*)-one (**11**) was accomplished in a 40% yield, using sodium *m*-nitrobenzene sulfonate in a sodium hydroxide solution. The unsubstituted derivatives **3g** and **12** had already been described in the literature and were synthesized according to the

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Table II. Cardiovascular Effects for **2**, **3**, **11**, and **12**

compd	antihypertensive activity ED_{50},[a] mg/kg po	rel act[b]	HR[c]	inotropic[d] activity
3b	3.50 (2.50–6.40)	1.17	+90	4.93 ± 0.05
(±)-**3c**	3.10 (1.67–5.40)	1.32	+90	5.54 ± 0.04
(+)-**3c**	2.10 (0.52–4.88)	1.95	+55	5.65 ± 0.09
3g	23.5 (18.2–32.7)	–	+70	4.98 ± 0.01
11	3.60 (1.50–5.64)	1.14	+50	5.77 ± 0.08
12	>25	–	−35	4.74 ± 0.11
2b	11.0 (6.52–14.62)	0.37	+49	5.05 ± 0.06
2c	7.10 (3.50–12.82)	0.58	+53	5.95 ± 0.02
dihydralazine	4.10 (3.50–4.80)	1.00	+68	
aminophylline				5.91 ± 0.03

[a] Dose that lowered the blood pressure by 50 mmHg in conscious spontaneously hypertensive rats (SHR) (peak effect). In parentheses are confidence limits for $P = 0.05$. [b] Relative activity based on dihydralazine (ED_{50} dihydralazine/ED_{50} compound). [c] Heart-rate variation (peak effect) at the highest used oral dose (25 mg/kg). [d] Evaluated "in vitro" on guinea pig spontaneously beating atria. Expressed as mean pD_2 (±SD) after cumulative administration starting from 2×10^{-8} M, according to J. M. Van Rossum.[22]

Table III. Other Biological Activities

compd	LD_{50}, mg/kg (mouse) po	LD_{50}, mg/kg (mouse) ip	antithrombotic[a] activity (mouse) po	antiaggregation activity in vitro[b] ADP, 0.5 μM revers	ADP, 0.5 μM irrevers	thrombin, 0.625 IU/mL 2 min	thrombin, 0.625 IU/mL 4 min	collagen, 0.8 μg/mL
3a	>1000	560	inactive	NT[c]		NT		NT
3b	>1000	611	73	10	42	13	27	21
(±)-**3c**	>1000	460	100	47	92	65	66	47
(+)-**3c**	>1000	>1000	89	36	77	91	100	2
(−)-**3c**	>1000	>1000	84	37	76	62	63	10
3d	>1000	>1000	33	NT		NT		NT
3e	>1000	378	inactive	inactive		NT		NT
3f	>1000	>1000	25	0	6	17	14	62
3g	666	450	59	30	95	84	97	100
11	>1000	651	55	40	95	81	86	5
12	>1000	707	33	NT		NT		NT
4b	700	483	45	NT		NT		NT
4c	>1000	>1000	46	NT		NT		NT
ASA	>1000	495	44	0	100	0	0	75

[a] Protection vs control percent: dose equimolar to 20 mg/kg ASA. [b] Guinea pig PRP is used. The compounds are tested at 66 μM (see the Experimental Section). Inhibition vs control percent. Mean of five experiments; SD < 20%. [c] Not tested.

reported methods.[10] See Table I for the physical properties of **6e,f**, **7e,f**, **9c**, and **10c**.

Results and Discussion

Compounds **3a–g**, **11**, and **12** were evaluated for their antihypertensive, inotropic, antithrombotic, and platelet aggregation inhibiting activities. Their antiinflammatory and antiulcer properties were also assessed. Compounds **4b,c** were tested for their antihypertensive and antithrombotic activities. Results are shown in Tables II and III.

The antihypertensive activity is reported as the dose which lowers by 50 mmHg (ED_{50}) the blood pressure of spontaneously hypertensive rats (SHR) (peak effect), with dihydralazine as the reference drug. The inotropic activity was studied "in vitro" on the guinea pig spontaneously beating atria. The force of contraction was recorded with an isometric transducer. Test compounds were administered cumulatively starting from a 2×10^{-8} M concentration, with aminophylline 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 [collagen (200 μg/mL) and adrenalin (200 μM)] and is reported as percent protection vs control at doses equimolar to 20 mg/kg of acetylsalicylic acid (ASA). In vitro platelet aggregation inhibiting activity of compounds effective as antithrombotic agents in vivo was performed with guinea pig platelet-rich plasma (PRP) preincubated with the test compounds at doses equimolar to 66 μM ASA. Aggregation was induced by adenosine diphosphate (ADP), thrombin, or collagen. The antiinflammatory activity was determined in the rat by carrageenin-induced edema of the hind paw. The antiulcer activity was evaluated in the rat by indomethacin-induced gastric lesions.

Compounds **3b**, (±)-**3c**, (+)-**3c**, **3g**, **11**, and **12** induced linear dose-dependent reductions of 20–80 mmHg in systolic blood pressure of SHR; the effect was rapid in onset and persisted for at least 6 h after medication (see Figure 1).

Compounds (±)-**3c**, (+)-**3c**, and **11** also caused the sharp drop of blood pressure exhibited with administration of dihydralazine and, in terms of relative activity, were more active than the reference drug (Table II). The heart rate (peak effect) at 25 mg/kg po seemed to be related to the antihypertensive activity, though not directly proportional to it. For example, compounds **11** and (+)-**3c** were, respectively, equipotent to and more active than (±)-**3c** as antihypertensive agents, but they induced lower heart-rate increases with respect to (±)-**3c**. By contrast, compound **12** exhibited bradycardic properties, whereas (−)-**3c** induced lethal tachycardic effects. Compounds **3a**, **3d**, **4b**, and **4c** were devoid of antihypertensive properties, whereas **3e** and **3f** showed a moderate, short-lasting antihypertensive activity that was not dose-related. The compounds listed in Table II were found to increase the guinea pig atria contractility and their activity in terms of pD_2 was comparable to that of aminophylline.

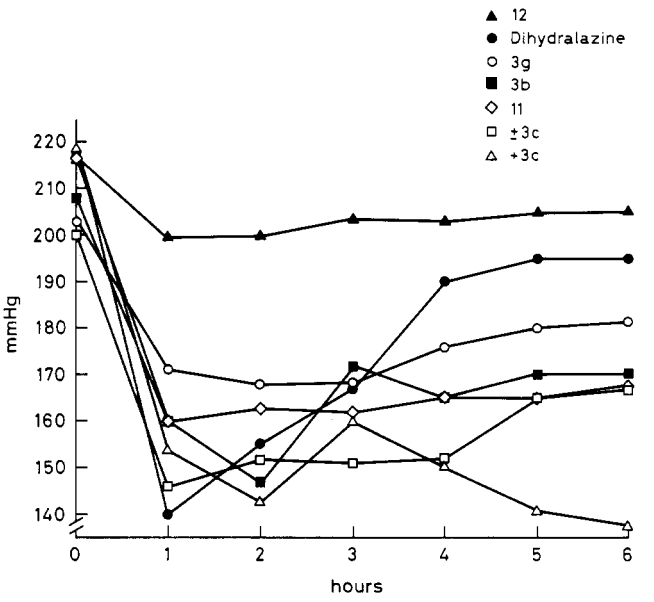

Figure 1. Time-dependent antihypertensive activity of **3b**, (±)-**3c**, (+)-**3c**, **3g**, **11**, **12**, and dihydralazine. Test compounds and reference drug are administered po at a single dose of 12.5 mg/kg to spontaneously hypertensive rats (groups of six animals).

Compounds **3b**, (±)-**3c**, (+)-**3c**, (−)-**3c**, **3g**, and **11** displayed antithrombotic properties greater than those of ASA, while **4b** and **4c** were equipotent with respect to the reference drug. The first series of antithrombotic agents was evaluated for platelet aggregation inhibiting activity. Compounds **3g** and (±)-**3c**, were found to inhibit aggregation induced by ADP, thrombin, or collagen, while (+)-**3c**, (−)-**3c**, and **11** only inhibited aggregation induced by ADP and thrombin. However, the antiaggregating

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activity of these compounds seems to involve a mechanism of action different from that of ASA and NSAID, which inhibit mainly the collagen-induced aggregation and the second phase curve of ADP-induced aggregation.[11] An hypothesis could be made that the compounds act by increasing the cellular level of cAMP through inhibition of cAMP–phosphodiesterase or that they increase prostacyclin production.[12,13]

Unlike the lower homologues **2**, none of the compounds of series **3** and **4**, exhibited antiinflammatory or antiulcer activity comparable to those of ASA and ranitidine, taken respectively as reference drugs. Antiinflammatory and antiulcer activities were tested according to the method of Winter et al.[14] and of Lee et al.,[15] respectively.

Among the compounds tested, the benzocinnolinones **3** presented the most interesting pharmacological profile, exhibiting marked analogies with the lower homologues **2**. In particular, not only did the 8-amino (**3b**) and the 8-acetylamino (**3c**) derivatives retain the antihypertensive and antithrombotic properties of their counterparts **2b** and **2c** but also their antihypertensive potency, in terms of relative activity based on dihydralazine, was respectively about three fold and two fold that of the corresponding derivative **2**. In addition, the dextro isomer (+)-**3c** was more active and showed a lower tachycardic effect than the racemic form. Only the 8-[(2-chloropropionyl)amino] derivative **3d** failed to parallel the profile of the related **2d**, displaying no antihypertensive properties and being weakly active as an antithrombotic agent. Unexpectedly, compound **11**, the aromatic analogue of **3c**, exhibited an antihypertensive potency which was comparable to the parent compound, in contrast to the loss in activity reported for aromatization of dihydropyridazinones **1**.[1] On the other hand, aromatization of **3g** to give **12** reduced both antihypertensive and antithrombotic properties. It is interesting to note that both **3g** and **12** lack the aromatic 8-acetylamino group, which seems to be a structural requirement for the biological activity of this class of compounds. The shift of the 8-acetylamino group of **3c** to the adjacent position 7 (**3e**) or 9 (**3f**) led to less active compounds, paralleling similar findings obtained for the indenopyridazinone homologues.[16]

The expansion of the central ring of **3b,c** to benzocycloheptapyridazinones **4b,c** caused a loss of antihypertensive activity and a marked decrease of the antithrombotic properties.

Compounds **3b,c,g**, **11**, and **12**, as well as the lower homologues **2b,c**, all active in lowering blood pressure, also displayed a positive inotropic action on guinea pig atria which roughly paralleled their antihypertensive potency. This brought us to infer that the two pharmacological effects could be referred to a similar mechanism. Since the cardiotonic properties of a series of derivatives of **2**, (including **2c**) and of **3c** had been found to mainly reside in phosphodiesterase (PDE) III inhibitory activity,[6] this mechanism was also likely to operate for our series of compounds. On this basis, it was of interest to verify whether the structural features of tricyclic pyridazinones **2–4** could be adapated to the five-point model proposed by Bristol and co-workers[17] to account for the activity of several noncatecholamine, nonglycoside cardiotonics which appear to exert their inotropic effects by inhibition of PDE III. Briefly, this model involves "(1) a strong dipole (carbonyl) at one end of the molecule, (2) an adjacent acidic proton, (3) a methyl-sized lipophilic space, (4) a relatively flat overall topography, and (5) a basic or hydrogen-bond acceptor opposite the dipole".

Subsequent SAR and X-ray studies[18] showed that this model is valid for dihydroarylpyridazinones (**1**). In the homologous series **2b,c**, **3b,c**, and **4b,c**, the structural features 1, 2, and 5 of the model were shared by all derivatives, whereas only **2b,c**, having a rigid planar structure, seemed to meet requirement 4. However, computer-derived energy minimization (MM2(85))[19] on the more flexible structures **3** and **4** revealed that, in the largely preferred conformation, **3** exhibited a near-planar arrangement of the pyridazinonic imino double bond and aromatic ring with a torsional angle N1–C10b–C10a–C10 of +4°. For the higher homologue **4**, a torsional angle N1–C11b–C11a–C11 of +31° was found, thus indicating a marked deviation from planarity.[20]

Since compounds **3b,c** are highly potent antihypertensive agents, while **4b,c** are inactive, the data presently available strongly suggest that an almost planar structure (point 4 of the model) is an essential requirement for the activity.

On the contrary, the methyl-sized space (point 3) seems to be of questionable importance for optimum activity of tricyclic pyridazinones, as shown by the higher potency of **3b,c** with respect to **2b,c**, in spite of the increased number of aliphatic carbons in the central ring. These results contrasted with the observed decrease in activity in aryldihydropyridazinones **1** when the size of the 5-substituent was increased from methyl to ethyl.[4,21] In addition, the minor contribution, if any, of this requirement to the activity of **2** and **3** could be highlighted by the observation that the lipophilic pocket of the receptor, which is able to fit the pseudoaxial 5-methyl group of **1**, could hardly accomodate the pseudoequatorial 5-methylene and 5,6-ethylene groups of **2** and **3**, respectively.

In conclusion, the Bristol model may not be completely applicable to the rigid congeners of aryldihydropyridazinones, thus supporting the hypothesis that the biological activities of compounds **2–4** are likely to be explained by peculiar structural features of the tricyclic system.

Experimental Section

Chemistry. Melting points were determined with a Büchi 510 capillary melting point apparatus and are uncorrected. The elemental analyses (C, H, N, and Cl) for the new substances were within ±0.4% of the theoretical values. IR spectra were recorded on a Perkin-Elmer 1310 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R 600 FT spectrophotometer, with tetramethylsilane as an internal standard.

6-(Acetylamino)-1,2,3,4-tetrahydro-1-oxo-2-naphthaleneacetic Acid (6c). To an ice-cooled solution of 6-(acetyl-

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amino)tetralone (**5c**)[7] (5 g, 0.024 mol) and glyoxylic acid (6.79 g, 0.091 mol) in water (90 mL) was added dropwise a solution of NaOH (3.5 g, 0.09 mol) in water (180 mL). The mixture was stirred at room temperature for 3 h and then acidified with concentrated HCl to pH 2. The solid which precipitated was filtered off, washed with water, and dried to give **6c** (5.1 g, 80%). A sample crystallized from ethanol melted at 235–37 °C. Anal. ($C_{14}H_{13}NO_4$) C, H, N.

Following the same procedure compounds **6e,f** were prepared starting from 5-(acetylamino)tetralone[8] and 7-(acetylamino)tetralone,[9] respectively (see Table I).

6-(Acetylamino)-1,2,3,4-tetrahydro-1-oxonaphthalene-2-acetic Acid (7c). A stirred mixture of **6c** (5 g, 0.019 mol), 40 mL of acetic acid, 15 mL of water, and 3.5 g of zinc dust was heated on a steam bath for 0.5 h and then filtered and diluted with water (300 mL). The cooled solution was extracted three times with ether (100 mL), the organic layer was dried with sodium sulfate, and the solvent was evaporated. The residue was recrystallized from ethanol to give **7c** (3.5 g, 71%), mp 195–97 °C. Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Following the same procedure, compounds **7e,f** were prepared (see Table I).

7-(Acetylamino)-2,3,4,5-tetrahydro-1-oxo-1*H*-2-benzocycloheptenideneacetic Acid (9c). Compound **9c** was prepared starting from the known **8c**[7] according to the method previously reported for **6c** (see Table I).

2-(Acetylamino)-benzosuberone-6-acetic Acid (10c). Compound **10c** was prepared by reduction of **9c** following the method previously reported for **7c** (see Table I).

Resolution of (±)-6-(Acetylamino)-1,2,3,4-tetrahydro-1-oxonaphthalene-2-acetic Acid (7c). To a hot solution of (±)-**7c** (5.8 g, 0.022 mol) in ethanol (87 mL) was added dropwise a solution of (+)-α-methylbenzylamine (2.78 g, 0.022 mol) in ethanol (30 mL). The solution was then left at room temperature for 2 h. The crystals which precipitated were filtered off and recrystallized twice from ethanol to give 4.0 g of a salt melting at 195 °C, $[\alpha]^{20}_D$ = +23.8° (DMSO). No change of the specific rotation was observed after an additional recrystallization. Anal. ($C_{22}H_{26}N_2O_4$) C, H, N. The salt was then dissolved in water and treated, on cooling, with 4 N HCl to give 2.3 g of (+)-**7c** as a white solid: mp 195–97 °C; $[\alpha]^{20}_D$ = +23.2° (DMSO). Anal. ($C_{14}H_{15}NO_4$) C, H, N. The mother liquors were evaporated to dryness and the solid residue (3.8 g) was treated with 4 N HCl to give 1.9 g of **7c** enriched in the levo isomer. Treatment with (−)-α-methylbenzylamine in ethanol gave the salt from which, following the above reported workup, 1.2 g of (−)-**7c** was obtained: mp 195–97 °C; $[\alpha]^{20}_D$ = −22.8° (DMSO). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

(±)-8-(Acetylamino)-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2*H*)-one (3c). A solution of **7c** (5 g, 0.019 mol) and hydrazine hydrate (1.1 g, 0.022 mol) in 100 mL of ethanol was refluxed for 3 h. After cooling, the product was filtered off, washed with ethanol, and dried to give **3c** (4.5 g, 91%), mp 287–90 °C. Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

Following the same procedure, compounds **3e** and **3f** were prepared from **7e** and **7f**, respectively. For **3e**: yield 60%; mp 158–160 °C. Anal. ($C_{14}H_{15}N_3O_2$) C, H, N. For **3f**: yield 68%; mp 185–87 °C. Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

4,4a,5,6-Tetrahydrobenzo[*h*]cinnolin-3(2*H*)-one (3g). Compound **3g** was prepared according to the literature,[10] mp 197–99 °C ($CHCl_3$) (lit.[10] mp 199–201 °C).

(+)-8-(Acetylamino)-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2*H*)-one ((+)-3c). The dextro isomer (+)-**3c** was prepared according to the method above reported for the racemic mixture, starting from (−)-**7c**: yield 84%; mp 284–85 °C; $[\alpha]^{20}_D$ = +158° (DMSO). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

(−)-8-(Acetylamino)-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2*H*)-one ((−)-3c). The levo isomer (−)-**3c** was prepared according to above reported method, starting from (+)-**7c**: yield 85%; mp 284–85 °C; $[\alpha]^{20}_D$ = −153° (DMSO). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

8-Amino-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2*H*)-one (3b). A suspension of **3c** (5 g, 0.019 mol) in 4 N HCl (75 mL) was heated at 100 °C for 2 h. After cooling, the mixture was brought to pH 4 by addition of 30% NaOH and the solid which precipitated was filtered off, washed with water, and dried to give **3b** (3.5 g, 85%). A sample crystallized from DMF melted at 277–80 °C. Anal. ($C_{12}H_{13}N_3O$) C, H, N.

8-Cyano-4,4a,5,6-tetrahydrobenzo[*h*]cinnolin-3(2*H*)-one (3a). To a cold, stirred solution of **3b** (6 g, 0.028 mol), in 12 mL of H_2O containing 7.2 mL of concentrated HCl, a cold solution of $NaNO_2$ (2.04 g, 0.029 mol) in 7.2 mL of water was added dropwise. The solution was carefully neutralized with Na_2CO_3 and then added in several portions to a cold, stirred solution of CuCN (3.72 g, 0.04 mol) and KCN (5.76 g, 0.088 mol) in 84 mL of water, which was covered with 25 mL of diethyl ether. The ice bath was removed, the mixture was allowed to reach room temperature, and then it was heated at 50 °C for 1 h. The solid, which separated after cooling, was filtered off, washed with a solution of KCN (1/10 w/v), and dried to give **3a** (5.2 g, 83%), mp 300 °C. Anal. ($C_{13}H_{11}N_3O$) C, H, N.

8-[(2-Chloropropionyl)amino]-4,4a,5,6-tetrahydrobenzo-[*h*]cinnolin-3(2*H*)-one (3d). 2-Chloropropionyl chloride (3.65 g, 0.029 mol) was added dropwise to a solution of **3b** (5 g, 0.023 mol) in 60 mL of absolute toluene. The mixture was then refluxed for 6 h, cooled, and filtered. The solid thus obtained was washed first with toluene and then with 5% $NaHCO_3$ solution to give **3d** (5.1 g, 72%). Anal. ($C_{15}H_{16}ClN_3O_2$) C, H, Cl, N.

8-(Acetylamino)-5,6-dihydrobenzo[*h*]cinnolin-3(2*H*)-one (11). A mixture of (±)-**3c** (1.5 g, 0.006 mol), sodium *m*-nitrobenzenesulfonate (1.4 g, 0.006 mol), NaOH (1.0 g, 0.025 mol), and 56 mL of water was refluxed for 1 h. After cooling, the resulting brown solution was acidified with 6 N HCl and the precipitate which formed was filtered off and triturated with ether to give **11** (0.8 g, 40%), mp 297 °C. Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

5,6-Dihydrobenzo[*h*]cinnolin-3(2*H*)-one (12). Compound **12** was prepared according to the literature,[10] mp 262–64 °C (EtOH) (lit.[10] mp 257–61 °C).

9-(Acetylamino)-2,4,4a,5,6,7-hexahydro-3*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazin-3-one (4c). A suspension of **10c** (2.8 g, 0.01 mol) and hydrazine hydrate (0.59 g, 0.012 mol) in 40 mL of ethanol was refluxed for 3 h. After cooling, the solid was filtered off and crystallized from ethanol to give **4c** (2.4 g, 87%), mp 253–55 °C. Anal. ($C_{15}H_{17}N_3O_2$) C, H, N.

9-Amino-2,4,4a,5,6,7-hexahydro-3*H*-benzo[6,7]cyclohepta-[1,2-*c*]pyridazin-3-one (4b). A suspension of **4c** (2 g, 0.007 mol) in 30 mL of 6 N HCl was refluxed for 2 h. After cooling, the solution was brought to pH 7 with 30% NaOH and the solid which formed was filtered off and washed throughly with water to give **4b** (1.2 g, 71%), mp 217–19 °C (EtOH). Anal. ($C_{13}H_{15}N_3O$) C, H, N.

Pharmacology. Antihypertensive Activity. Experiments were performed on unanesthetized SH 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 six animals per 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 gavage at dose levels of 1.56, 3.125, 6.25, 12.5, and 25 mg/kg. Systolic blood pressure was recorded every hour for 3 h after drug administration. ED_{50} values were calculated from the log dose–response curves. Dihydralazine was used as a standard drug.

Positive Inotropic Activity. Positive inotropic activity was assessed with spontaneously beating guinea pig atria. The atria of the freshly sacrificed guinea pig were isolated and suspended in Krebs–Ringer solution at 32 °C in an organ bath of 50-mL capacity. The tissue was aerated with a gaseous mixture of 95% oxygen and 5% carbon dioxide. The force of contraction was recorded through an isometric transducer (Grass Model FT 0.03). An initial tension of 750 mg was given to the preparation. Stabilization time for the preparation was 60 min. After the basal response was taken, the test compounds were administered on a cumulative basis, starting from 2×10^{-8} M. A contact time of 7 min was given for each dose. pD_2 values were calculated from dose–response curves, according to Van Rossum.[22] Aminophylline was used as a standard drug; its pD_2 value was 5.9 ± 0.03.

Platelet Aggregation Inhibiting Activity in Vitro. The determination was carried out by the method described by Born

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and Cross.[23] Male crossbreed guinea pigs weighing 350–500 g, 18-h fasted, were used. Under sodium pentobarbital narcosis, blood was taken from the abdominal aorta and was rendered nonclotting by adding a 3.8% (w/v) sodium citrate solution (final volume ratio 1:10). A plasma rich in platelets was then obtained as supernatant by centrifuging. Aggregation was triggered by adding (a) ADP at doses ranging from 0.25 to 2 μM/mL, (b) collagen (from equine tendon) at doses ranging from 0.8 to 2.4 μg/mL, and (c) thrombin at doses ranging from 0.312 to 1.25 units/mL. Incubation of the platelets with the test compounds was carried out for 10 min at room temperature at a dose equimolar to the minimal dose of ASA (66 μM dissolved in 0.3 M CH_3COONa) which completely inhibits platelet aggregation. The inhibiting action was expressed as percent of inhibition by comparing the aggregation curve of the test compound with that of the control.

Antithrombotic Activity in Vivo. The determination was carried out by a modification of the method of Minno and Silver.[24] Male Swiss mice weighing 20–30 g were divided in three groups of 10. Groups 1 and 2 were treated with test compounds and the reference drug (ASA), respectively, both dissolved in 1% (hydroxymethyl)cellulose and orally administered in a volume of 50 mL/kg. The dose of the test compound was equimolar to that of ASA (20 mg/kg). One hour after medication, groups 1 and 2, along with group 3 (controls), received a thrombotic mixture [fetal bovine collagen (200 μg/mL) and adrenaline (200 μM)] in a volume of 10 mL/kg administered iv in the tail (before injection animals were warmed at 27 °C for 30 min). Death of the animals or paralysis for more than 15 min of the hind limbs were considered as thrombotic effects. The antithrombotic activity was characterized as percent protection (%P) by relating the number of the thrombotic effects in group 1 (treated) to those of group 3 (controls), according to the following formula: $\%P = (N_c - N_t)/N_c \times 100$. The protection of the test compound was then compared to that of the reference drug (group 2).

Registry No. (±)-**3a**, 121866-02-2; (±)-**3b**, 103603-13-0; (±)-**3c**, 121866-03-3; (+)-**3c**, 103602-97-7; (−)-**3c**, 103602-95-5; **3d**, 121866-04-4; (±)-**3e**, 110138-94-8; (±)-**3f**, 110138-93-7; (±)-**3g**, 121866-05-5; (±)-**4b**, 103603-08-3; (±)-**4c**, 103602-79-5; **5c**, 88611-67-0; **5e**, 102873-24-5; **5f**, 58161-21-0; **6c**, 103602-85-3; **6e**, 110139-16-7; **6f**, 110139-13-4; (±)-**7c**, 103602-78-4; (+)-**7c**, 103602-89-7; (+)-**7c** (+)-α-methylbenzylamine salt, 103602-90-0; (−)-**7c**, 103602-91-1; (±)-**7e**, 110139-07-6; (±)-**7f**, 110139-08-7; **9c**, 103602-86-4; (±)-**10c**, 121866-01-1; **11**, 104120-90-3; **12**, 25823-49-8; glyoxylic acid, 298-12-4; (+)-α-methylbenzylamine, 3886-69-9; (−)-α-methylbenzylamine, 2627-86-3; 2-chloropropionyl chloride, 7623-09-8.

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Novel Benzodiazepine Receptor Partial Agonists: Oxadiazolylimidazobenzodiazepines

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The synthesis and biochemical evaluation of a series of oxadiazole derivatives of imidazobenzodiazepines related to the benzodiazepine antagonist Ro 15-1788 (**2a**) are reported. Although the oxadiazole ring is seen as an isosteric replacement for the ester linkage, significant differences in structure–activity trends were observed. Specifically, oxadiazoles **9–12** invariably had increased receptor efficacy (as witnessed by measurements of the GABA shift) relative to the corresponding ester. Additionally, and in direct contrast to the classical agonists such as diazepam, affinity for the benzodiazepine receptor was enhanced by a 7- rather than 8-halo substituent. The results are discussed in terms of a six-point receptor-binding model originally based on the X-ray structure of **2a**. For comparison, the crystal structures of two representative oxadiazole derivatives, **10h** and **12o**, having a 6-oxo and 6-phenyl group, respectively, were determined and the data incorporated into a modified binding model to account for the greater efficacy of these compounds. It is concluded that the antagonist behavior of **2a** relies upon the hydrogen-bond-acceptor properties of the ester carbonyl oxygen whereas for the oxadiazole series this site is localized at the imidazole nitrogen.

It is well-established that benzodiazepines and related ligands interact with a specific site ("the benzodiazepine receptor") that is closely associated with a neuro-inhibitory, postsynaptic $GABA_A$ receptor and a chloride ionophore channel.[1,2] The efficiency of coupling of the $GABA_A$ receptor to the chloride ion effector mechanism can be modified by several series of compounds that bind at this site. Uniquely, it has been shown that this receptor can be occupied by ligands having a continuum of intrinsic efficacy, from positive efficacy (anxiolytic, anticonvulsant, and sedative agents), through nil intrinsic efficacy (receptor antagonists) to negative intrinsic efficacy (proconvulsant, anxiogenic agents).[3,4] The existence of these three categories would also imply that partial agonists and partial inverse agonists exist. Partial inverse agonists may be useful as cognition enhancers.[5] This spectrum of differing efficacy has been most clearly demonstrated in the β-carbolines[4,6] 1 and, more recently, in the imidazobenzodiazepine series, **2**.[7,8]

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