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Benzofurazanyl- and benzofuroxanyl-1,4-dihydropyridines: synthesis, structure and calcium entry blocker activity

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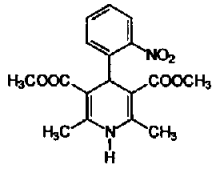
Summary — The synthesis, structural characterization and calcium blocking activity of a series of benzofurazanyl-1,4-dihydropyridines (**18** and **19**) and benzofuroxanyl analogues (**20** and **21**) are reported. ^{1}H-NMR showed that all the benzofuroxan derivatives exist in solution as tautomeric mixtures. The predominant tautomeric form in solution of the derivative **20** (dimethyl 1,4-dihydro-2,6-dimethyl-4-(4-benzofuroxanyl)-3,5-pyridinedicarboxylate) is also the one preferred in the solid state as shown by X-ray analysis. The conformation in the solid state of the benzofurazanyl analogue is also reported. Calcium entry blocker activity of the dihydropyridine derivatives **18–21** has been evaluated in isolated rabbit basilar artery as relaxation of calcium-induced contractions in high K^+-depolarizing solution. All the compounds displayed high potency. The activity of benzofurazan derivatives was not changed by the *N*-oxidation. The two most active compounds **18** and **20** were as potent as Nifedipine.

calcium channel blocker / benzofuroxanyl-1,4-dihydropyridine / benzofurazanyl-1,4-dihydropyridine / benzofuroxan tautomerism / X-ray analysis

Introduction

4-Aryl-1,4-dihydropyridines (4-aryl-DHPs) form a major class of drugs used in the management of cardiovascular diseases [1, 2]. Nifedipine (dimethyl 1,4-dihydro-2,6-dimethyl-4-(*o*-nitrophenyl)-3,5-pyridinedicarboxylate) **1** is the prototype of this class and has been object of several structural modifications.



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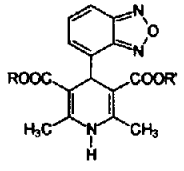

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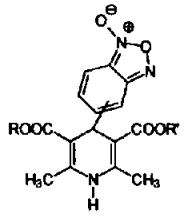

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Important Nifedipine analogues are derivatives **2** in which a benzofurazan system is present at the 4-position of the 1,4-dihydropyridine ring [3]. Among these compounds Isradipine (**2**, R = CH_3, R' = $CH(CH_3)_2$) has received particular attention. This drug displays selective effects on coronary arteries and the sinus node, but not on atrioventricular conduction [4].

In our laboratory we have recently begun a study on the benzofuroxan (benzofurazan *N*-oxide) derivatives **3**, analogues of Nifedipine. In this paper we report the synthesis, structural characterization and calcium channel blocking activities of derivatives **18–21** in which the 1,4-dihydropyridine moiety is linked to the benzofurazanyl and benzofuroxanyl substructures.

Chemistry

The classical synthetic approach to obtain 1,4-dihydropyridine calcium channel blockers (DHPs) is based upon the Hantzsch procedure [5]. This reaction implies the cyclization of an aldehyde, a dicarbonyl compound and ammonia. Several modifications of this synthesis have been reported and among them the cyclization of an aldehyde with 2 mol of β-aminocrotonic ester is particularly useful [6]. We used this procedure for the preparation of the final compounds,

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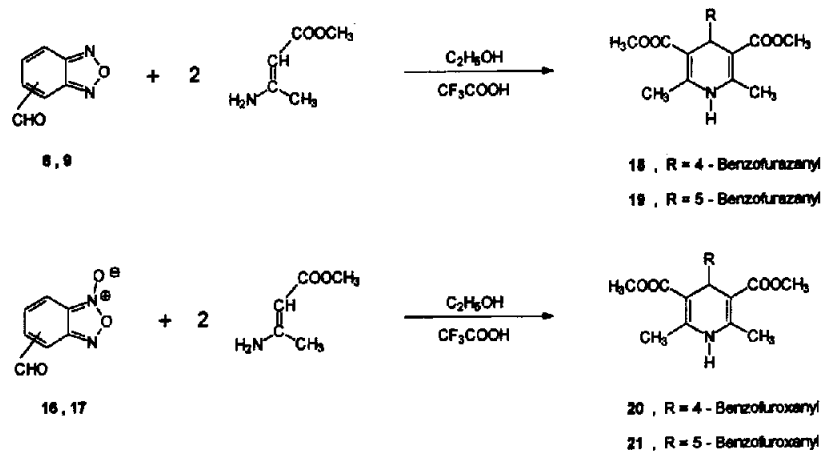
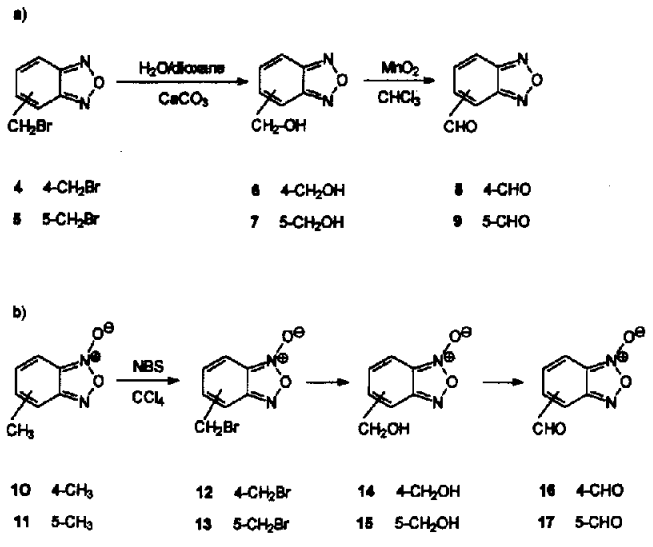


Scheme 1.



Scheme 2.

employing benzofurazan and benzofuroxan aldehydes and β-aminocrotonic methyl ester dissolved in absolute ethanol for the cyclization, in presence of trifluoroacetic acid (scheme 1).

The starting aldehydes **8** and **9** used to obtain the benzofurazan derivatives **18** and **19** are described in reference [7] where they were prepared from the corresponding bromomethylbenzofurazans by the Sommelet reaction. We synthesized them according to the pathway reported in scheme 2a.

4-Bromomethylbenzofurazan **4** and its 5-isomer **5** were hydrolyzed in a 1:1 mixture of water/dioxane in the presence of calcium carbonate. The alcohols **6** and **7** obtained were oxidized in good yields to the corresponding aldehydes **8** and **9** by active manganese dioxide in chloroform solution. The ^{1}H-NMR spectra (table I) are in keeping with the proposed structures.

The syntheses and structures of benzofuroxan derivatives require a more detailed discussion. It is known that monosubstituted benzofuroxan derivatives exhibit the tautomeric rearrangement **I** ⇌ **II**.

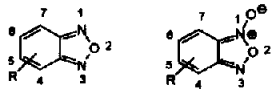
I **II**

The effects of a number of R-substituents on the position of the equilibrium are known [8]. According to a convention [9], when a tautomeric pair of compounds is being considered, it is referred to by assigning the lowest numerical value to the substituent.

The intermediate 4-formylbenzofuroxan **16** [10] has been described previously in literature. It was obtained by refluxing 2-azido-3-nitrobenzaldehyde for 5 h in toluene. In these conditions **16** is in rapid tautomeric equilibrium with 7-nitroanthranil. The isolation of the compound formed is tedious and time-consuming. We prepared **16** according to the pathway depicted in scheme 2b. By action of *N*-bromosuccinimide on 4-methylbenzofuroxan **10** dissolved in carbon tetrachloride solution, in the presence of a catalytic amount of benzoyl peroxide, the corresponding 4-bromomethyl derivative **12** was obtained. This compound was hydrolyzed in the same way as that reported for the benzofurazan analogue. The 4-hydroxymethyl derivative **14** was oxidized to the 4-formyl derivative **16** by action of active manganese dioxide.

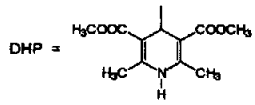
Compound **10** in chloroform at room temperature is in equilibrium with the 7-CH_3 isomer, as shown by broad peaks due to incomplete coalescence (4-CH_3/7-CH_3 ratio 75:25; 100 MHz NMR detection at –44°C

Table I. ^{1}H-NMR data of the benzofurazan and benzofuroxan derivatives **4–21**.



4-9, 18, 19

10-17, 20, 21



Compound	*R*	*Temperature (°C)*[a]	*Percentage of tautomer*	*Ring protons*[b]	*R protons*[b]
10[c]	4-CH_3	–44	75	7.15–7.23	2 61
	7-CH_3		25	6.94–7.93	2.60
11[c]	5-CH_3	–46	52	7.10–7.35	2.46
	6-CH_3		48	7.13–7.55	2.44
4	4-CH_2Br			7.68–8.10	5.11
5	5-CH_2Br			7.60–8.11	4.81
12	4-CH_2Br	–10	75	7.4–7.9	5.05
	7-CH_2Br		25	7.4–7.9	4.98
13	5-CH_2Br	–20	45	7.51–7.95	4.87
	6-CH_2Br		55	7.68–7.90	4.87
6	4-CH_2OH			7.63–7.98	4.99 (CH_2); 5.75 (OH)
7	5-CH_2OH			7.51–7.98	4.64 (CH_2); 5.64 (OH)
14	4-CH_2OH	–10	70	7.3–7.7	5.05 (CH_2); 5.29 (OH)
	7-CH_2OH		30	7.3–7.7	5.05 (CH_2); 5.17 (OH)
15	5-CH_2OH	–10	50	7.36–7.63	4.82; 5.20
	6-CH_2OH		50	7.42–7.64	4.80; 5.20
8	4-CHO			7.96–8.52	10.35
9	5-CHO			7.88–8.85	10.13
16	4-CHO	–10	93	8.00–8.40	10.39
	7-CHO[d]		7	–[d]	10.63
17	5-CHO	–10	25	7.75–8.65	10.25
	6-CHO		75	7.79–8.43	10.18
18	4-DHP			7.26–7.80	DHP[e]
19	5-DHP			7.50–7.94	DHP[e]
20	4-DHP	–10	82	7.3–7.6	DHP[e]
	7-DHP		18	7.3–7.6	DHP[e]
21	5-DHP	–20	50	7.1–7.8	DHP[e]
	6-DHP		50	7.1–7.8	DHP[e]

[a]Temperature of the determination of the equilibria; [b] δ, ppm from TMS, solvent CD_3COCD_3; [c]data taken from lit [9], solvent $CDCl_3$; [d]ring proton resonances missing due to the very low abundance of this tautomer; [e]the resonances relative to the dihydropyridine moiety are in keeping with those reported in the literature for similar derivatives.

[9]). In acetone solution at room temperature **12** shows a 200 MHz ^{1}H-NMR spectrum with broad resonance signals. The broad signal related to CH_2Br group is resolved at –10°C into two narrow peaks (4-CH_2Br/7-CH_2Br ratio 75:25; table I). A similar situation occurs with the alcoholic derivative **14** for which the ratio between the 4-CH_2OH/7-CH_2OH isomers measured at –10°C was about 70:30. The NMR spectrum of the aldehyde **16** at temperatures in the range –40°C to 35°C gave quite narrow peaks showing that neither the benzofuroxan nor the anthranilic tautomerism was important. Electronic and steric effects cause the equilibrium to be strongly biased towards the 4-isomer. A similar situation occurs for the final compound **20**. The X-ray analysis of the crystalline tautomeric form, which is usually the predominant form in solution [8], is also in agreement (fig 1b).

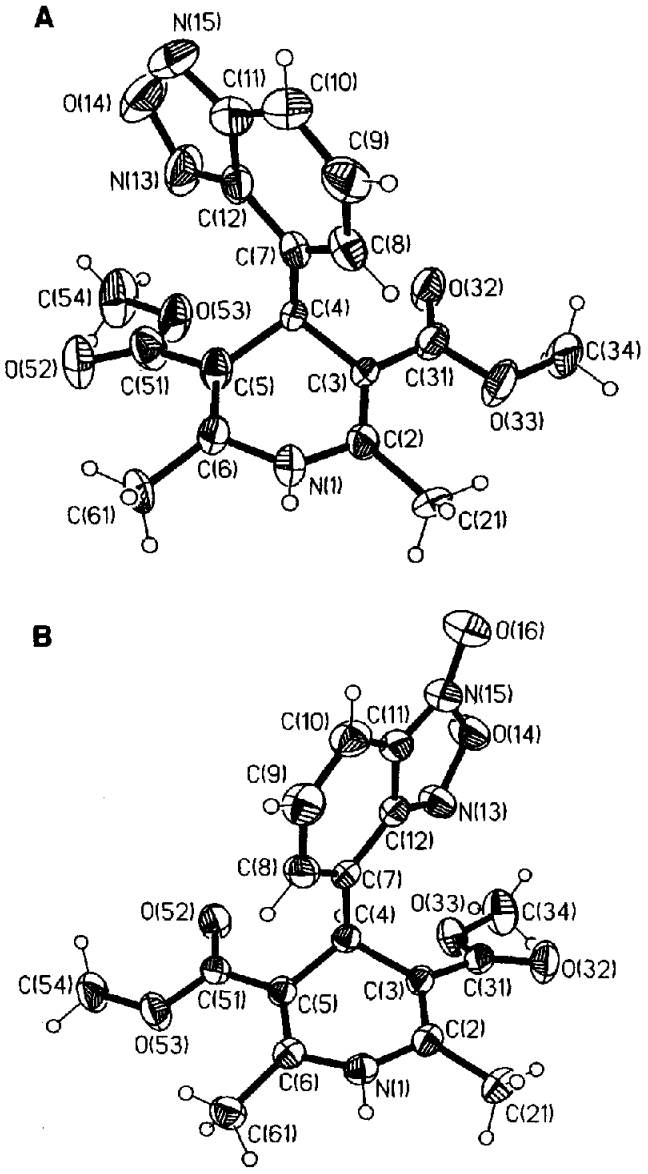


Fig 1. Ortep drawing of **18** (**A**) and **20** (**B**) showing thermal ellipsoids.

The intermediate 5-formylbenzofuroxan **17**, found on the way to **21**, has also been described [11]. It was prepared by heating a mixture of 4-chloro-3-nitrobenzaldehyde and sodium azide in DMSO at 75°C. We obtained this compound by using the same procedure as that reported for **16** (scheme 2b).

In the benzofuroxan 5-substituted series all the ^{1}H-NMR spectra show very broad peaks indicating extensive benzofuroxan tautomerism. At low temperature the study of the ratio between 5- and 6-isomers is possible (table I). For the final derivative **21** this ratio was about 1:1 at –20°C in acetone solution. A detailed NMR study of the benzofuroxans in this work will be published later.

Crystal structures

The solid state structures for the derivatives **18** and **20** are reported in figure 1, which shows the thermal ellipsoids and atom-numbering schemes. We report only some of their features here.

The bond lengths and angles in the benzofurazan and benzofuroxan moieties are in keeping with the available data [12] on X-ray diffraction studies of derivatives of these two heterocycles. In particular the two systems are planar and the benzofuroxan shows the characteristic features of this ring, namely an unusually short N(15)-O(16) bond (1.23 Å) and a rather long N(15)-O(14) bond (1.44 Å). The DHP rings are in flattened boat conformations. The distortion of the dihydropyridine system from planarity as reflected by $\Sigma[\tau]$, the sum of absolute values of the six ring torsion angles, is 135.3° for **18** and 126.0° for **20**. Both the furazan and furoxan moieties are positioned towards the C(4)-hydrogen atom of the DHP ring (synperiplanar position). The benzofurazan substructure is directed towards the C(5)-methoxycarbonyl group while the benzofuroxan one is directed towards the C(3)-methoxycarbonyl function. The value of the C(8)-C(7)-C(4)-C(3) torsion angle Φ, which determines the conformation about the inter-ring bond, is –11.1° in **18** and –108.5° in **20**. The closer this torsion angle is to –60°, the closer the benzo-fused system comes to bisecting the DHP ring. The ester groups are rotated about the bonds C(3)-C(31) (torsion angle C(2)-C(3)-C(31)-O(32)) and C(5)-C(51) (torsion angle C(6)-C(5)-C(51)-O(52)) respectively of 173.5° and 29.4° in **18** and –35.0° and –174.8° in **20**. The methoxycarbonyl function at C(3) carbon shows an *ap* conformation and the one at C(5) carbon an *sp* conformation in **18**, while in **20** the situation is the reverse.

Pharmacological results and discussion

Calcium entry blocker activity of the dihydropyridine derivatives **18**–**21** has been evaluated in isolated rabbit basilar artery as relaxation of calcium-induced contractions in high K^+-depolarizing solution. All the derivatives tested at a range of concentrations between 0.1 to 1000 nM induced a concentration-dependent relaxing effect (fig 2).

The two most active products were **18** and **20** in which the 1,4-dihydropyridine ring is linked to the 4-position of the benzoheterocyclic system. They show the same potency (**18**, IC_{50} = 2.8 (1.85–4.1) nM;

20, IC_{50} = 2.0 (1.4–2.9) nM) as the Nifedipine taken as reference (IC_{50} = 1.1 (0.7–1.5) nM).

The pair of compounds **19** and **21** in which the 1,4-dihydropyridine ring is linked to the 5-position of the benzoheterocyclic system are about tenfold less potent (**19**, IC_{50} = 28.5 (14.9–59.6) nM; **21**, IC_{50} = 19.4 (10.6–39.1) nM).

Recently, it has been shown that several furoxan derivatives display strong vasodilating activities owing to their ability to release nitrogen oxide under the action of thiol cofactors and consequently to activate the soluble guanylate cyclase [13, 14]. This is not the case of the derivatives **20** and **21**, which, at concentration < 10^{-5} M, were found unable to activate, above the basal level, the guanylate cyclase present in RFL-6 cells, a rat fibroblast cell line whose guanylate cyclase is particulary sensitive to NO.

In conclusion the *N*-oxidation does not modify the range and the ratio of activity of the benzofurazanyl-1,4-DHPs or the mechanism of their vasorelaxing action.

Experimental protocols

Chemistry

Melting points were recorded on a Buchi 530 capillary melting point apparatus and are uncorrected. All the compounds were routinely checked by IR spectroscopy (Perkin-Elmer Model 781) and mass spectrometry (Finningan-Mat TSQ-700). ^{1}H-NMR spectra (data shown in table I) were recorded on a Bruker AC-200 spectrometer. Silica gel (Merck Kieselgel 100) 70-230 mesh ASTM was employed for column chromatography. Petroleum ether (bp 40–60°C) was used for the chromatographic purifications and crystallizations. Anhydrous magnesium sulfate was used as drying agent. Derivatives **4, 5** [7] and **10, 11** [15] were synthesized according to the literature procedures. The elemental analyses for new compounds were performed by Redox (Cologno M). Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values.

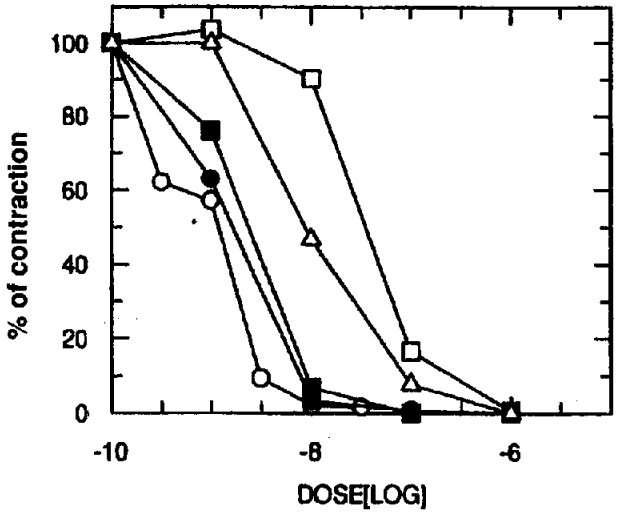


Fig 2. Effect of derivatives **18–21** on contractions evoked by Ca^{2+} in K^+-depolarized rabbit basilar artery. ○ **1**, ● **20**, ■ **18**, △ **21**, □ **19**.

*Preparation of bromomethylbenzofuroxans **12** and **13**. General procedure*

To a stirred solution of the appropriate methylbenzofuroxan derivative (7.51 g, 50 mmol) in dry carbon tetrachloride (100 ml), were added *N*-bromosuccinimide (9.80 g, 55 mmol) and a catalytic amount of benzoyl peroxide. The mixture was refluxed under stirring for 4 h and then filtered. The residue obtained after *in vacuo* solvent removal was purified by crystallization to afford the pure title compounds.

4-Bromomethylbenzofuroxan (**I** ⇌ **II**, R = CH_2Br) **12**, 82% yield, mp 94–95°C (chloroform/petroleum ether). Anal $C_7H_5BrN_2O_2$ (C, H, N). 5-Bromomethylbenzofuroxan (**I** ⇌ **II**, R = CH_2Br) **13**, 75% yield, mp 75°C (ethanol). Anal $C_7H_5BrN_2O_2$ (C, H, N).

*Preparation of hydroxymethylbenzofurazans **6** and **7** and hydroxymethylbenzofuroxans **14** and **15**. General procedure*

To a stirred solution of the appropriate bromomethyl derivative (30 mmol) in dioxane (80 ml), calcium carbonate (15.01 g, 150 mmol) and water (80 ml) were added. The mixture was refluxed for 3 h under stirring and then evaporated *in vacuo*. The residue was treated with methylene chloride and then with 2 N hydrochloric acid until dissolution of the white precipitate occurred. The separated aqueous phase was extracted with methylene chloride. The combined organic layers, dried and evaporated *in vacuo*, gave a residue which was purified on a short silica-gel column (eluent: petroleum ether/ethyl acetate 70:30) to afford the pure title products.

4-Hydroxymethylbenzofurazan **6**, 77% yield, mp 73°C (chloroform/petroleum ether). Anal $C_7H_6N_2O_2$ (C, H, N). 5-Hydroxymethylbenzofurazan **7**, 74% yield, mp 52–53°C (chloroform/petroleum ether). Anal $C_7H_6N_2O_2$ (C, H, N). 4-Hydroxymethylbenzofuroxan (**I** ⇌ **II**, R = CH_2OH) **14**, 85% yield, mp 88–89°C (chloroform/petroleum ether). Anal $C_7H_6N_2O_3$ (C, H, N). 5-Hydroxymethylbenzofuroxan (**I** ⇌ **II**, R = CH_2OH) **15**, 75%, mp 56–57°C (diisopropylether/petroleum ether). Anal $C_7H_6N_2O_3$ (C, H, N).

*Preparation of formylbenzofurazans **8** and **9** and formylbenzofuroxans **16** and **17**. General procedure*

To a stirred solution of the appropriate alcohol (10 mmol) in chloroform (100 ml), activated manganese dioxide (8 g) was added. The mixture was vigorously stirred at room temperature for 2 h and then filtered through Celite. The filtrate was concentrated *in vacuo* to give the pure expected compounds.

4-Formylbenzofurazan **8**, 92% yield, mp 108–109°C, lit [7], mp 108–109°C (petroleum ether). 5-Formylbenzofurazan **9**, 91%, mp 57–58°C, lit [7], mp 57–58°C (petroleum ether/a few drops of chloroform). 4-Formylbenzofuroxan (**I** ⇌ **II**, R = CHO) **16**, 93%, mp 102°C dec (ethyl acetate/petroleum ether), lit [10], mp 102°C dec. 5-Formylbenzofuroxan (**I** ⇌ **II**, R = CHO) **17**, 92% yield, mp 68–69°C (ethyl acetate/petroleum ether), lit [11], mp 68–69°C.

Table II. Atomic coordinates (x 10^4) and equivalent isotropic displacement coefficients (Å^2 x 10^3) for compound **18**[a].

	x	*y*	*z*	*U(eq)*[b]
N(1)	3909(9)	1674(5)	10110(4)	44(3)
C(2)	5365(12)	1216(6)	10560(5)	37(4)
C(3)	7053(12)	1429(6)	10376(5)	31(3)
C(4)	7265(11)	2252(7)	9768(5)	33(3)
C(5)	5760(12)	2298(6)	9074(5)	35(3)
C(6)	4117(13)	2114(6)	9314(5)	40(4)
C(7)	7128(12)	3200(7)	10249(6)	39(4)
C(8)	6548(13)	3360(7)	11080(6)	44(4)
C(9)	6337(14)	4249(9)	11493(6)	54(4)
C(10)	6764(14)	5038(7)	11097(7)	53(4)
C(11)	7365(13)	4920(8)	10226(7)	50(4)
C(12)	7553(12)	4028(7)	9812(6)	37(4)
N(13)	8161(11)	4109(6)	9018(5)	60(4)
O(14)	8328(10)	5066(6)	8942(5)	68(3)
N(15)	7885(12)	5574(6)	9709(6)	66(4)
C(21)	4862(12)	488(6)	11178(5)	40(4)
C(31)	8764(14)	983(6)	10750(6)	41(4)
O(32)	10249(8)	1145(5)	10526(4)	55(3)
O(33)	8534(8)	379(5)	11401(4)	61(3)
C(34)	10149(13)	–73(7)	11809(7)	68(5)
C(51)	6172(14)	2537(7)	8175(6)	47(4)
O(52)	5094(9)	2930(5)	7665(4)	61(3)
O(53)	7896(9)	2217(4)	7979(3)	50(3)
C(54)	8490(14)	2409(8)	7114(6)	71(5)
C(61)	2414(12)	2231(7)	8790(6)	50(4)
N(1')	6089(9)	3327(5)	5114(4)	39(3)
C(2')	5888(13)	2896(6)	4313(6)	40(4)
C(3')	4216(11)	2693(6)	4078(5)	34(3)
C(4')	2766(11)	2749(6)	4767(5)	32(3)
C(5')	2947(11)	3561(6)	5379(5)	35(4)
C(6')	4605(12)	3798(7)	5560(5)	38(4)
C(7')	2899(11)	1799(7)	5247(6)	35(4)
C(8')	3469(12)	1660(7)	6075(6)	46(4)
C(9')	3654(14)	752(9)	6492(6)	58(5)
C(10')	3251(15)	–32(8)	6101(7)	62(5)
C(11')	2624(13)	71(8)	5238(7)	48(4)
C(12')	2454(11)	977(7)	4826(6)	36(4)
N(13')	2155(12)	–571(7)	4704(6)	70(4)
O(14')	1665(10)	–71(6)	3945(5)	74(3)
N(15')	1865(11)	878(7)	4022(5)	58(4)
C(21')	7625(12)	2763(8)	3794(6)	56(4)
C(31')	3854(13)	2458(6)	3189(6)	35(4)
O(32')	2109(9)	2785(4)	2977(3)	48(3)
O(33')	4870(9)	2069(5)	2664(4)	63(3)
C(34')	1539(15)	2591(7)	2112(6)	63(4)
O(53')	1453(8)	4620(5)	6396(4)	57(3)
O(52')	–245(9)	3857(5)	5529(4)	54(3)
C(51')	1245(14)	4022(7)	5743(6)	42(4)
C(54')	–142(13)	5069(8)	6794(6)	69(5)
C(61')	5130(12)	4514(7)	6186(6)	50(4)

[a]Two independent molecules are present in the cell but only one is represented in figure 1a. [b]Equivalent isotropic *U* defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table III. Atomic coordinates (x 10^4) and equivalent isotropic displacement coefficients (Å^2 x 10^3) for compound **20**.

	x	*y*	*z*	*U(eq)*
N(1)	11147(2)	–90(1)	1639(1)	32(1)
C(2)	10961(3)	702(1)	2062(2)	31(1)
C(3)	9309(3)	919(1)	2268(2)	29(1)
C(4)	7837(3)	222(1)	2198(2)	27(1)
C(5)	8002(3)	–384(1)	1408(2)	28(1)
C(6)	9684(3)	–552(1)	1188(2)	29(1)
C(7)	7993(3)	–252(1)	3127(2)	29(1)
C(8)	8609(3)	–1078(2)	3280(2)	38(1)
C(9)	8786(4)	–1495(2)	4168(2)	49(1)
C(10)	8308(4)	–1104(2)	4920(2)	44(1)
C(11)	7655(3)	–250(2)	4781(2)	36(1)
C(12)	7498(3)	181(1)	3921(2)	31(1)
C(13)	6863(3)	976(1)	3965(1)	42(1)
O(14)	6567(3)	1085(1)	4866(1)	51(1)
N(15)	7079(3)	296(1)	5373(1)	43(1)
O(16)	6873(3)	270(1)	6190(1)	64(1)
C(21)	12658(3)	1245(2)	2186(2)	43(1)
C(31)	8906(3)	1805(2)	2533(2)	34(1)
O(32)	9959(2)	2301(1)	2979(1)	51(1)
O(33)	7169(2)	2024(1)	2202(1)	41(1)
C(34)	6556(4)	2856(2)	2465(2)	58(1)
C(51)	6282(3)	–771(1)	962(2)	30(1)
O(52)	4800(2)	–561(1)	1135(1)	48(1)
O(53)	6473(2)	–1394(1)	351(1)	47(1)
C(54)	4820(4)	–1804(2)	–95(2)	57(1)
C(61)	10177(3)	–1175(2)	482(2)	38(1)

*Preparation of benzofurazany-1,4-dihydropyridines **18** and **19** and benzofuroxanyl-1,4-dihydropyridines **20** and **21**. General procedure*

To a stirred and ice-salt cooled (*ca* –10°C) solution of the appropriate benzofurazancarbaldehyde (5 mmol) in absolute ethanol (10 ml), trifluoroacetic acid (1.14 g, 10 mmol) and, dropwise, a solution of methyl aminocrotonate (1.73 g, 15 mmol) in absolute ethanol (10 ml) were sequentially added. The reaction mixture was kept under stirring at 0°C for 1.5 h. The pure precipitate formed was filtered, washed with a small amount of cold ethanol and dried. In the preparation of **19** an additional crop of material was obtained by adding 1 N sodium bicarbonate (50 ml) to the filtered solution, by extracting with ethyl acetate and then by *in vacuo* solvent removal and crystallization of the residue.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-(4-benzofurazanyl)-3,5-pyridinedicarboxylate **18**, 70% yield, mp 218–221°C dec (tetrahydrofuran/petroleum ether). Anal $C_{17}H_{17}N_3O_5$ (C, H, N). Dimethyl 1,4-dihydro-2,6-dimethyl-4-(5-benzofurazanyl)-3,5-pyridinedicarboxylate **19**, 68% yield, mp 172–173°C (tetrahydrofuran/petroleum ether). Anal $C_{17}H_{17}N_3O_5$ (C, H, N).

Table IV. Crystal data for compounds **18** and **20.**

	Compound 18	*Compound 20*
Crystal data		
Empirical formula	$C_{17}H_{17}N_3O_5$	$C_{17}H_{17}N_3O_6$
Color habit	Yellow prisms	Yellow prism
Crystal size (mm)	0.13 x 0.18 x 0.30	0.37 x 0.37 x 0.18
Crystal system	Triclinic	Monoclinic
Space group	*P*-1	*P*21/*c*
Unit cell dimensions	a = 7.480(2) Å b = 14.253(3) Å c = 15.394(3) Å α = 88.63(3)° β = 89.80(3)° γ = 82.81(3)°	a = 7.410(3) Å b = 15.414(5) Å c = 14.630(6) Å β = 99.14(2)°
Volume	1627.9(8) Å^3	1649.8(11) Å^3
Z	4	4
Formula weight	343.3	359.3
Density (calc)	1.401 mg/m^3	1.447 mg/m^3
Absorption coefficient	0.105 mm^{-1}	0.112 mm^{-1}
F(000)	720	752
Data collection		
Diffractometer	Siemens P4	Siemens P4
Radiation	Mo$K\alpha$ (λ = 0.71069 Å)	Mo$K\alpha$ (λ = 0.71069 Å)
Temperature (K)	293	298
Monochromator	Highly oriented graphite crystal	Highly oriented graphite crystal
2θ range	7.0–45.0°	7.0–50.0°
Scan type	ω	ω
Scan speed	Variable; 2.00–29.30°/min in ω	Variable; 2.00–15.00°/min. in ω
Scan range (ω)	0.06°	1.00°
Background measurement	Stationary crystal and stationary counter at beginning and end of scan, each for 25.0% of total scan time	stationary crystal and stationary counter at beginning and end of scan, each for 35.0% of total scan time
Standard reflections	2 measured every 50 reflections	2 measured every 50 reflections
Index ranges	$-8 < h < 8, -15 < k < 15$ $0 < l < 16$	$-8 < h < 8, 0 < k < 18$ $0 < l < 17$
Reflections collected	4424	3118
Independent reflections	4235 (R_{int} = 11.06%)	2929 (R_{int} = 1.39%)
Observed reflections	2118 ($F > 4.06(F)$)	2041 ($F > 4.06(F)$)
Absorption correction	N/A	N/A
Solution and refinement		
System used (Siemens)	SHELXTL IRIS [16]	SHELXTL IRIS [16]
Solution	Direct methods (SIR92 program [17])	Direct methods (SIR92 program [17])
Refinement	Full-matrix least-squares	Full-matrix least-squares
Quantity minimized	$\Sigma w(F_o\text{-}F_c)^2$	$\Sigma w(F_o\text{-}F_c)^2$
Absolute structure	N/A	N/A
Extinction correction	N/A	N/A
Hydrogen atoms	Riding model, fixed isotropic U	Riding model, fixed isotropic U
Weighting scheme	$w^{-1} = \sigma^2(F) + 0.0018F^2$	$w^{-1} = \sigma^2(F) + 0.0006F^2$
Number of parameters refined	451	237
Final R indices (obs data)	R = 7.07%, wR = 9.33%	R = 4.08%, wR = 4.69%
R indices (all data)	R = 16.41%, wR = 13.51%	R = 6.67%, wR = 5.18%
Goodness-of-fit	1.63	1.26
Largest and mean Δ/σ	0.006, 0.001	0.001, 0.000
Data-to-parameter ratio	4.7:1	8.6:1
Largest difference peak	0.33 eÅ^{-3}	0.20 eÅ^{-3}
Largest difference hole	–0.34 eÅ^{-3}	–0.20 eÅ^{-3}

Dimethyl 1,4-dihydro-2,6-dimethyl-4-(4-benzofuroxanyl)-3,5-pyridinedicarboxylate (**I** ⇌ **II**, R = 4-DHP) **20**, 80% yield, 156–164°C dec (tetrahydrofuran/petroleum ether) Anal $C_{17}H_{17}N_3O_6$ (C, H, N). Dimethyl 1,4-dihydro-2,6-dimethyl-4-(5-benzofuroxanyl)-3,5-pyridinedicarboxylate (**I** ⇌ **II**, R = 5-DHP) **21**, 88% yield, mp 176–177°C dec (ethyl acetate/petroleum ether). Anal $C_{17}H_{17}N_3O_6$ (C, H, N).

Pharmacology

Male New Zealand albino rabbits (2.9–3.2 kg) were anaesthetized with sodium pentobarbital (60 mg/kg iv) and then sacrificed by exanguination from the femoral artery.

The basilar artery was quickly dissected and suspended as rings between two l-shaped stainless steel hooks in 20 ml organ baths containing a modified Ca^{2+}-free high K^+-depolarizing Krebs-Henseleit solution of the following composition (mM): NaCl 63, KCl 60, $MgCl_2$ 1.18, KH_2PO_4 1.17, $NaHCO_3$ 17, glucose 11.6.

The solution was gassed with 95% O_2, 5% CO_2 at pH 7.4 and maintained at 37°C. The initial resting tension applied was 0.5 g.

Developed tension was recorded by means of a Grass FT03 force transducer connected to a Battaglia Rangoni KV380 polygraph system.

After 45 min stabilization period, preparations were contracted by 1 mM $CaCl_2$; when contraction reached its maximum, a cumulative concentration–response curve (0.1–1000 nM) with the compounds **18–21** was carried out.

Each preparation received only one compound and at least six tissue preparations were used for each compound. Control tissues received vehicle only.

Structure determination

The final atomic coordinates for non-hydrogen atoms and the thermal parameters are listed in tables II and III. Bond lengths, angles, torsion angles, thermal parameters and structure factors are available. Crystal data for compounds **18** and **20** are given in table IV.

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