Organic Nitrates II[1]

Synthesis and Biological Activities of 4-Nitrooxymethylphenyl-1,4-dihydropyridines

Jochen Lehmann[a]*, Rainer Kahlich[a], Christoph Meyer zum Gottesberge[b], and Uwe Fricke[b]

[a] Pharmazeutisches Institut der Universität Bonn, An der Immenburg 4, D-53121 Bonn, Germany

[b] Institut für Pharmakologie der Universität zu Köln, Gleueler Str. 24 , D-50931 Köln, Germany

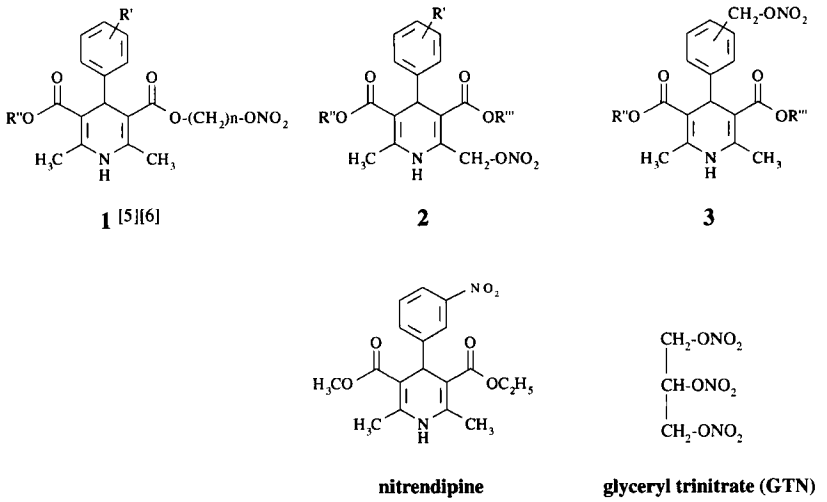
Key Words: *Organic nitrates; 1,4-dihydropyridines; calcium channel antagonists; nitrooxyaldehydes; vasodilators*

Summary

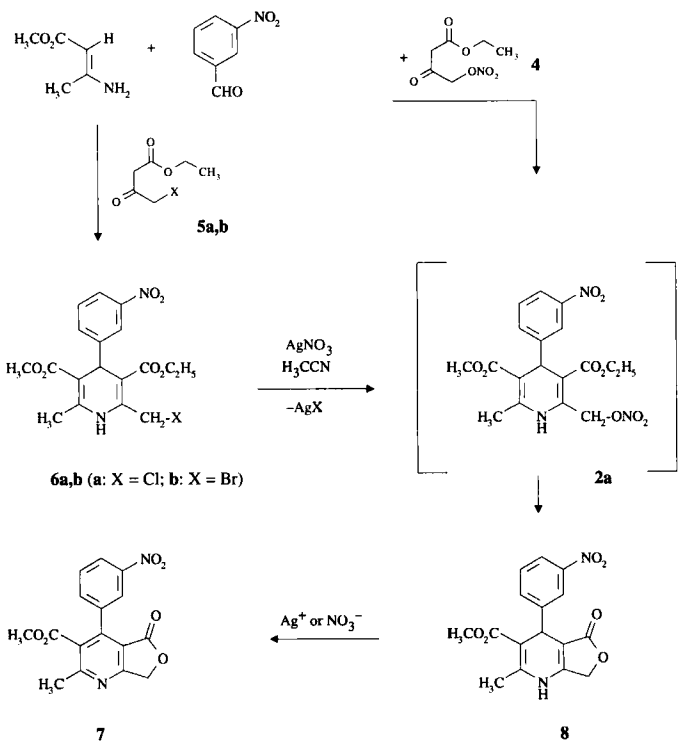
Both 2-nitrooxymethyl-4-phenyl- (**2**) and 4-nitrooxymethylphenyl-1,4-dihydropyridines (**3**) represent new combinations of two different vasodilating structures. **2** could not be isolated due to its spontaneous lactonization. Derivatives of **3** were obtained *via* Hantzsch synthesis using nitrooxymethylated benzaldehydes. The inotropic potency in isolated porcine trabecular muscles and the vasodilator activity in isolated porcine coronary arteries of four nitrooxyphenyl-dihydropyridines were determined. Nitrendipine (NTD) and glyceryl trinitrate (GTN) were used for reference. **3** were negative inotropic, however, less than NTD and – except for the dicyano derivative **3d** – more than GTN. Vasodilator properties were less pronounced than that of both nitrendipine and GTN. Vascular selectivity was low.

Introduction

1,4-Dihydropyridines such as nifedipine are calcium channel blockers with high affinity for the voltage-dependent L-type calcium channels, leading to potent and predominantly arterial vasodilation[2]. In addition, recent studies indicate at least for nitrendipine a release of nitric oxide from vascular endothelium[3], which may contribute to the vascular effects of the drug. Compared to the 1,4-dihydropyridines, organic nitrates like glyceryl trinitrate predominantly cause dilation of venous vessels[4], which is mainly deduced from nitric oxide liberation. Clinically, these drug actions lead to a decrease in cardiac preload (nitrates) and cardiac afterload (dihydropyridines) and both calcium channel blockers and organic nitrates may therefore be used in the treatment of angina pectoris and coronary heart disease as well as in arterial hypertension.



Scheme 1



Scheme 2

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1997 0365-6233/97/0808/0247 $17.50 +.50/0

Derivatives of **1** proved to be the first promising vasodilators, combining the properties of organic nitrates and calcium channel blockers[5, 6]. We now wish to report on investigations concerning the synthesis of **2** and **3**, both structures representing a new combination of the two different vasodilating principles, and some biological activities of **3.**

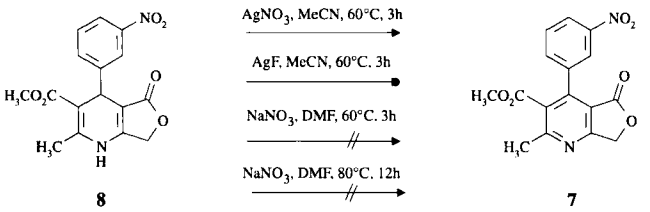
Results

Chemistry

Synthesis of the nitrooxylated nitrendepine **2a** should be performed by Hantzsch reaction using β-aminocrotonate, 3-nitrobenzaldehyde, and the γ-nitrooxyacetoacetate **4**, prepared from of the bromo derivative **5b** and silver nitrate. A minimum temperature of 45 °C was necessary to start any reaction but then **2a** could not be isolated due to its spontaneous lactonization yielding **8**.

Alternatively we tried to obtain **2a** from the halogenated dihydropyridines **6a,b**. These two compounds displayed a significant difference in reactivity. **6a** also needed elevated temperatures to undergo substitution and was treated with silver nitrate at 65 °C for 72 h. Again compound **8** was obtained. **6b** reacted at room temperature and was stirred with silver nitrate for 20 h. While stirring a mirror of elementary silver developed at the vessel wall and the sole product which could be isolated was the heteroaromatic derivative **7**.

In order to find out whether **8** was oxidized by Ag^+ or by NO_3^-, **8** was treated under argon with solutions of $AgNO_3$, AgF, and $NaNO_3$ with the following result:

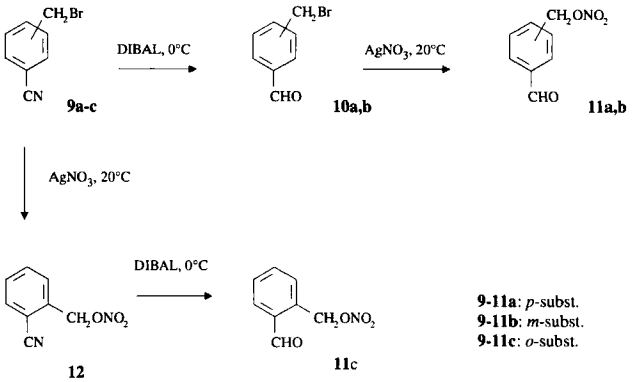


8 **7**

Scheme 3

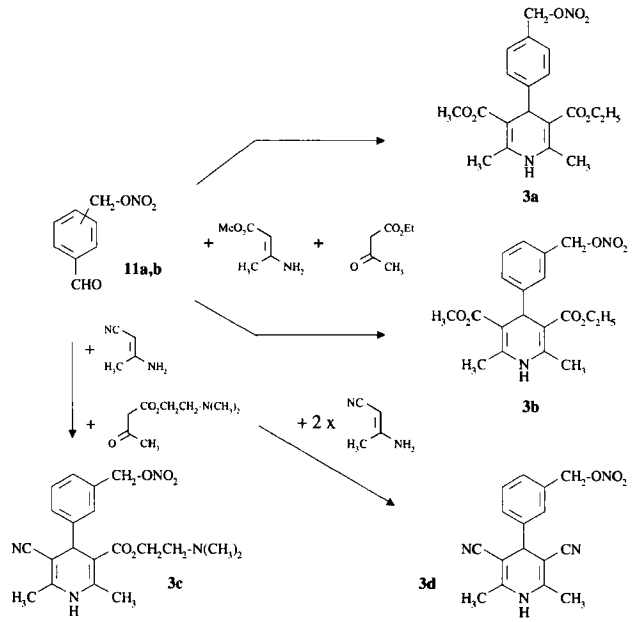
The two silver salts give **7** as sole product, $NaNO_3$ only reacts at > 80 °C and produces a small amount of **7** together with unreacted **8**. Thus Ag^+ is considered to be the aromatizing agent.

The preparation of the nitrooxymethylated dihydropyridines **3** by Hantzsch reaction afforded nitrooxymethylated benzaldehydes and the question was if one could synthesize such structures because there might be an immediate internal oxidation of the aldehyde by the nitrato group. According to this we only found one type of compound (β-lactam derivative) in the literature carrying both an aldehyde and a nitrato group in one molecule. We were able to synthesize the nitratoaldehydes **11a,b** by treatment of **10a,b** with silver nitrate according to Scheme 4 and they proved to be surprisingly stable. Since we failed to prepare **10c** following the same route, we tried to invert the two reaction steps for the preparation of this *o*-substituted aldehyde. Reduction of the nitrooxymethylnitrile **12** yielded a product which displayed the expected signals in the ^{1}H-NMR, but it was unstable, impure, and not suitable for any further reactions.



Scheme 4

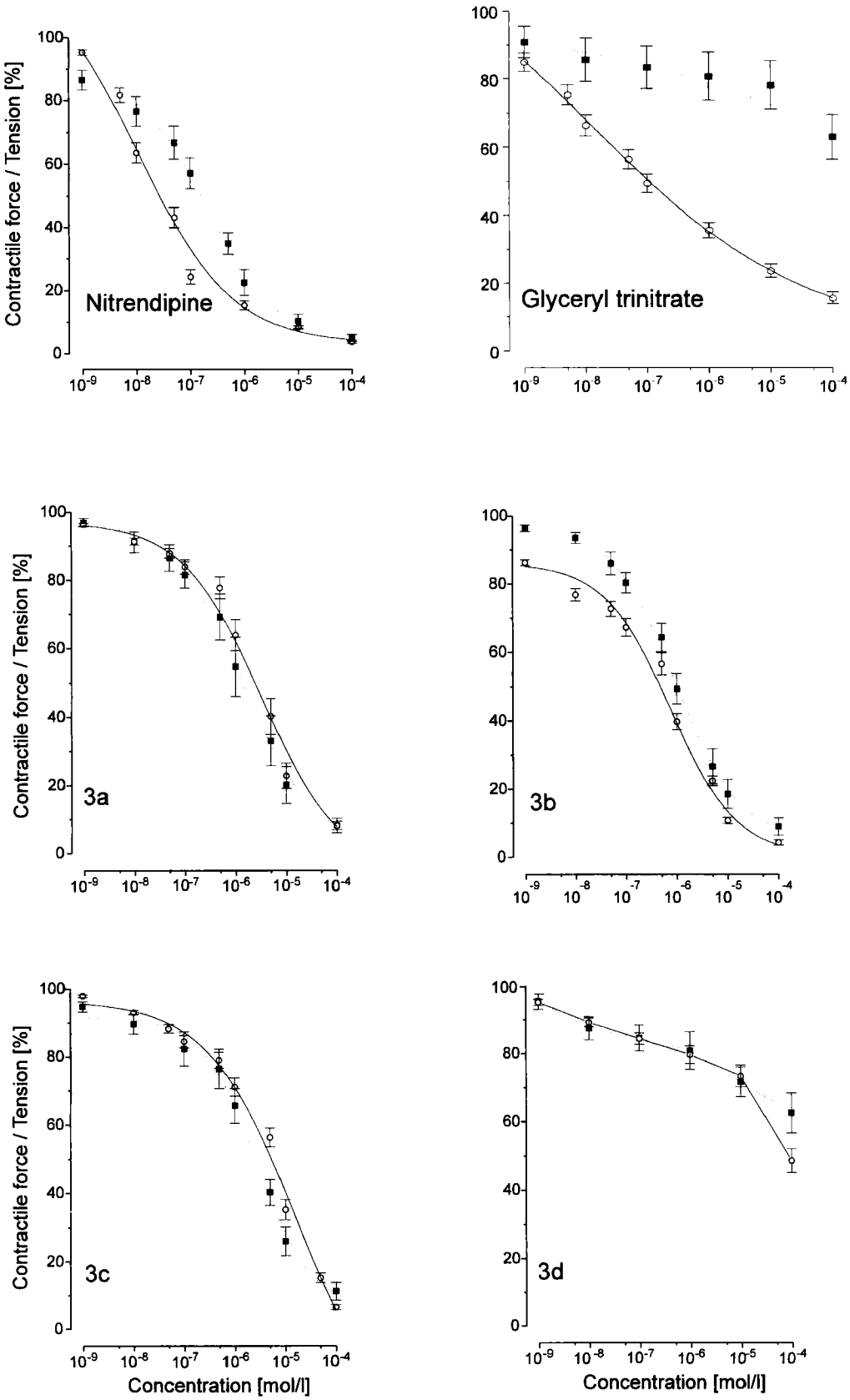
Hantzsch reactions, using the impure **11c** proved to be unsuccessful, but treatment of the *m*- and *p*-nitrooxymethylbenzaldehydes with ethyl acetoacetate and methyl β-aminocrotonate in acetonitrile at 35 °C gave **3a,b**. Compound **3c** was obtained from **11b**, methyl β-aminocrotononitrile, and dimethylaminoethyl acetoacetate and finally **3d** was synthesized from **11a** and two molecules of β-aminocrotononitrile. All target compounds were subjected to cc purification plus 2–3 recrystallizations and were isolated in poor yields (6–17%).



Scheme 5

Biological Activity

Inotropic activity: Both the dihydropyridines and glyceryl trinitrate (GTN) reduced the contractile force of stimulated porcine trabecular muscles (Scheme 6) in a dose-dependent way, the nitrooxymethylated **3** being considerably less active than nitrendipine (NTD). Least potent was **3d**, which lead to a reduction of the contraction amplitude by about 40% only at the highest concentration tested (Scheme 6). A comparable weak negative inotropic effect was obtained with GTN. Thus the negative inotropic potency increased in the following



Scheme 6. - -■- - Influence of increasing concentrations of the dihydropyridines and GTN (abscissa, mol/l) on contractile force of isolated porcine trabecular muscles (ordinate, % of control contractile force). Each dose-response-curve is plotted from the respective mean values of 5 to 7 individual experiments. —○— Influence of increasing concentrations of the dihydropyridines and GTN (abscissa, mol/l) on KCl (60 mmol/l) precontracted porcine coronary artery rings (ordinate, % of control contraction). Each dose-response-curve is plotted from the respective mean values of 5 to 8 individual experiments.

Table 1: Biological activities of the 1,4-dihydropyridines and glyceryl trinitrate.

Compound	Cardiodepression[1] EC50 [µmol/l]	*n*	Vasorelaxation[2] EC50 [nmol/l]	*n*	Vascular selectivity[3]
NTD	0.185 ± 0.04	6	30 ± 5.2	8	6.17
GTN	>> 100[4]	6	156 ± 58	5	> 600
3a	2.23 ± 0.99	6	2920 ± 770	8	0.76
3b	1.63 ± 0.77	7	497 ± 102	6	3.28
3c	2.75 ± 0.61	6	5070 ± 970	8	0.54
3d	>> 100[4]	5	>> 100 000	6	–

[1] Negative inotropic activity obtained in porcine trabecular muscles. [2] Vasodilator activities obtained in porcine coronary arteries, means and standard error of means (SEM) of n individual experiments are given. [3] Calculated from the concentrations for half maximal response in cardiac muscle and arterial vessels. [4] Extrapolated values.

order: GTN < **3d** << **3c** < **3a** < **3b** < NTD (Table 1). Corresponding EC_{50} values varied between 185 nmol/l (NTD) and more than 100 µmol/l (GTN and **3d**).

Vasodilator activity: All dihydropyridines and GTN produced a dose dependent inhibition of the KCl-induced contraction of porcine right coronary arteries (Scheme 6). Corresponding EC_{50} values were in the range of 30 nmol/l (NTD) and more than 100 µmol/l (**3d**), so vascular activity increased in the order: **3d** << **3c** < **3a** < **3b** < GTN < NTD (Table 1). Compared to NTD all nitrooxymethylated dihydropyridines were markedly less active, with **3d** leading to only half maximal vasorelaxation at the highest concentration tested (100 µmol/l).

Vascular selectivity: Vascular selectivity of the drugs studied can be expressed as the ratio of the half-maximal inotropic and the half-maximal vasodilator concentration (Table 1). Such calculated values increased in the following order: **3c** < **3a** < **3b** < NTD < GTN. Compared to NTD all tested nitrooxymethylated dihydropyridines exhibited a decreased vascular selectivity. The outstanding selectivity of GTN is mainly dependent on the very weak cardiodepressant activity of this drug (Table 1).

In contrast, the nitrates **3a** and **3c** rather show a slight cardiac preference. Because of the low potency of **3d**, the vascular selectivity of this compound could not be estimated.

Discussion

The results of our study reveal that nitrooxymethylation of the phenyl ring in 4-phenyl-1,4-dihydropyridines reduces cardiodepressant and arterial vasodilator activity compared to nitrendipine. The reason for this is supposed to be the "wrong" substitution pattern of these molecules. Both any substituent in the para position (compound **3a**) of the phenyl ring and replacement of the ester group in position 3 and 5 by other electron-withdrawing groups including -CN (compounds **3c,d**) cause a lower binding affinity at the L-type calcium channel, leading to reduced activity of these dihydropyridines[7]. In accordance to this **3b** proved to be the relatively most active of the nitrooxymethylated derivatives. All compounds **3** also lack a small electron withdrawing group (NO_2 or CF_3) in *m*- or *o*-position of the phenyl ring, comparable to the nitro group in nitrendipine. Finally it seems, that these compounds could be calcium antagonists with a comparatively low potency and vascular selectivity, the properties of which could be possibly improved by adjusting the substitution pattern. The character of any additional organic-nitrate-like effects must be clarified in further studies on isolated venous vessels.

Experimental Part

General

Melting point: uncorrected.– Elemetal analysis: Heraeus CHN-O-Rapid and Carlo Erba 1106 CHN-Analyser.– IR: Pye Unicam 3200S and Perkin-Elmer 1420.– ^{1}H-NMR: Bruker WH 90 (90 MHz) and Varian XL 300 (300 MHz), int. stand. TMS.– MS: Varian CH 7 and Kratos MS 50 Kratos (70eV).– Flash column chromatography: 42 × 9 cm column, flow 50 ml/min, 1.2–1.4 bar, argon; silica gel 0.04–0.06 mm (Baker); acetone/petroleum ether 1:3. Reactions involving dihydropyridines were performed in the dark or in vessels protected from light by aluminium foil. Dried equipment was used for reductions with DIBAL.

*Ethyl γ-nitrooxyacetoacetate (**4**)*

A solution of $AgNO_3$ (12.2 g, 72 mmol) in 40 ml acetonitrile was added dropwise to **5b** (15.0 g, 72 mmol) in 60 ml acetonitrile. The mixture was stirred for 2 h at 38 °C internal temp. AgBr was separated, the filtrate evaporated and the residue thoroughly washed with 3 × 100 ml water and extracted with 3 × 125 ml CH_2Cl_2. The combined extracts were dried (Na_2SO_4), evaporated and distilled. 2.34 g (12.1 %) slightly coloured oil, bp. 80–82 °C/0.1 torr.– IR (NaCl): = 1750, 1700, 1650, 1540, 1270 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): (ppm) = 1.25 (t, *J* = 6 Hz, 3H, CH_3), 3.43 (s, 2H, 2-CH_2), 4.00 (q, *J* = 6 Hz, 2H, OCH_2), 4.95 (s, 2H, O_2NOCH_2).– Anal. ($C_6H_9NO_6$).

*Ethyl methyl 2-chloromethyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (**6a**)*

3-Nitrobenzaldehyde (11.4 g, 75 mmol), methyl-β-aminocrotonate (8.6 g, 75 mmol) and **5a** (12.3 g, 75 mmol) were dissolved in warm EtOH (120 ml), protected from light and stirred under argon for 6 h at 47 °C. The mixture was evaporated and the orange residue purified by flash CC. Fractions 1–3 contained 3-nitrobenzaldehyde, fractions 4–8 **6a**, and fractions 9, 10 the lactone **8**. Fractions 4–8 were combined, evaporated and the residue recrystallized twice from methanol. 8.0 g (27 %) yellow crystals, mp 128–129 °C (ref.[8] 130–133 °C.– IR (KBr): ν = 3900, 3325, 2960, 1705, 1655, 1595, 1520, 1360 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.23 (t, *J* = 7 Hz, 3H, CH_2-*CH_3*), 2.36 (s, 3H, 6-CH_3), 3.65 (s, 3H, OCH_3), 4.11 (mc, 2H, *CH_2*-CH_3), 4.80, 4.97 (2 × **d**, *J* = 13.5 Hz, CH_2Cl), 5.13 (s, 1H, 4-H), 6.70 (s, 1H, NH), 7.39 (dd, *J* = 8, 8 Hz, 1H, 5′-H), 7.63 (ddd, *J* = 8, 2, 1 Hz, 1H, 6′-H), 8.01 (ddd, *J* = 8, 2, 1 Hz, 1H, 4′-H), 8.11 (dd, *J* = 2, 2 Hz, 1H, 2′-H).– Anal. ($C_{18}H_{19}N_2O_6Cl$).

*Ethyl methyl 2-bromomethyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (**6b**)*

Preparation and cc isolation (fractions 4–7) analog **6a**, using **5b** (15.8 g , 75 mmol). 1.4 g (7.3 %) yellow crystals, mp 105–107 °C.– IR (KBr): ν = 3350, 3100, 2970, 1705, 1650, 1575, 1550, 1350 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.24 (t, *J* = 7 Hz, 3H, CH_2-*CH_3*), 2.39 (s, 3H, 6-CH_3), 3.65 (s, 3H, OCH_3), 4.12 (mc, 2H, *CH_2*-CH_3), 4.63, 4.77 (2 × d, *J* = 11 Hz, CH_2Br), 5.15 (s, 1H, 4-H), 6.52 (s, 1H, NH), 7.39 (dd, *J* = 8, 8 Hz, 1H, 5′-H), 7.63 (ddd, *J* = 8, 2, 1 Hz, 1H, 6′-H), 8.01 (ddd, *J* = 8, 2, 1 Hz, 1H, 4′-H), 8.12 (dd, *J* = 2, 2 Hz, 1H, 2′-H).– Anal. ($C_{18}H_{19}N_2O_6Br$).

*Methyl 2-methyl-4-(3-nitrophenyl)-5-oxo-1,4,5,7-tetrahydro-furo[3,4-b]pyridine-3-carboxylate (**8**)*

3-Nitrobenzaldehyde (3.34 g, 22 mmol), methyl β-aminocrotonate (2.52 g, 22 mmol) and **4** (4.2 g, 22 mmol) were stirred in acetonitrile (60 ml) at 45 °C for 3 h.. The mixture was evaporated and the remaining brown oil dissolved in hot methanol. The solid precipitated in the cold was collected and recrystallized from methanol. 0.52 g (7.2 %) yellow powder, mp 230 °C (ref.[8] 238 °C).– IR (KBr): ν = 3300, 3090, 2980, 1740, 1700, 1660, 1570, 1350 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.46 (s, 3H, 6-CH_3), 3.58 (s, 3H, OCH_3), 4.71, 4.78 (2 × d, *J* = 16Hz, CH_2OCO), 5.05 (s, 1H, 4-H), 6.88 (s, 1H, NH), 7.46 (dd, *J* = 10, 7.5 Hz, 1H, 6′-H), 7.72 (ddd, *J* = 8, 2, 1 Hz, 1H, 4′-H), 8.05 (dd, *J* = 2.5, 1 Hz, 1H, 5′-H), 8.07 (dd, *J* = 2.5, 2 Hz, 1H, 2′-H).– Anal. ($C_{16}H_{14}N_2O_6$).

*Methyl 2-methyl-4-(3-nitrophenyl)-5-oxo-5,7-dihydro-furo[3,4-b]pyridine-3-carboxylate (**7**)*

A solution of $AgNO_3$ (0.17 g, 1.0 mmol) in acetonitrile (10 ml) was added dropwise to **6b** (0.44 g, 1.0 mmol) dissolved in acetonitrile (60 ml). After stirring at room temp. for 20 h, AgBr was separated and the filtrate evaporated. The oily residue was washed (3 × 100 ml water) and extracted 3× with CH_2Cl_2. The combined extracts were dried (Na_2SO_4), evaporated and the residue recrystallized twice from methanol. 190 mg (58 %) yellow needles, mp 208 °C (ref.[9] 200–202 °C).– IR (KBr): ν = 2960, 1770, 1720 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.75 (s, 3H, 6-CH_3), 3.67 (s, 3H, OCH_3), 5.33 (s, 2H, CH_2), 7.85 (mc, 4 H, aromat.).

*Bromomethyl-benzaldehydes **10a,b***

DIBAL (50 ml of a 1molar solution in hexane) was added dropwise under stirring at 0 °C during 20 min to bromomethylbenzonitrile (6.0 g, 30.6 mmol) in 60 ml chlorobenzene. Stirring at 0 °C was continued for 2.5 h. The mixture was diluted with $CHCl_3$ (100 ml), cautiously hydrolyzed with 150 ml 10% hydrochloric acid and extracted with $CHCl_3$. The combined extracts were dried (Na_2SO_4), and evaporated. The remaining oil crystallizes by addition of ether and hexane. Recrystallization from *n*-hexane.

*4-Bromomethyl-benzaldehyde (**10a**):* From **9a** 4.3 g (78 %) slightly coloured platelets, mp 98 °C (ref.[10] 94–96 °C.– IR (KBr): ν = 1695, 1170, 820, 790, 720 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.53 (s, 2H, CH_2), 7.55, 7.85 (AB-system, 4H, aromat.), 10.00 (s, 1H, CHO).

*3-Bromomethyl-benzaldehyde (**10b**):* From **9b** 4.05 g (66 %) white crystals, mp 47 °C (ref.[11] 46–49 °C.– IR (KBr): ν = 1700, 1205, 900, 800, 730, 700, 670 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.52 (s, 2H, CH_2), 7.70 (mc, 4H, aromat.), 9.98 (s, 1H, CHO).7.55, 7.85 (AB-system, 4H, aromat.).

*Nitrooxymethyl-benzaldehydes **11a,b***

A solution of $AgNO_3$ (2.5 g, 14.7 mmol) in 20 ml acetonitrile was added dropwise to **10** (2.6 g, 13 mmol) in 20 ml acetonitrile. The mixture was stirred at room temp. for 2 h. AgBr was separated, the filtrate evaporated and the residue thoroughly washed with 3 × 100 ml water and extracted with 3 × 125 ml CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and evaporated. The remaining oil crystallized on addition of ether and *n*-hexane. Recrystallization from *n*-hexane.

*4-Nitrooxymethyl-benzaldehyde (**11a**):* From **10a** 1.2 g (51 %) yellow needles, mp 41 °C.– IR (NaCl): ν = 1705, 1650, 1540, 1435, 1335, 1270, 1205, 1150, 825, 730 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 5.48 (s, 2H, CH_2), 7.55, 7.92 (AB-system, 4H, aromat.) 10.00 (s, 1H, CHO).– Anal. ($C_8H_7NO_4$).

*3-Nitrooxymethyl-benzaldehyde (**11b**):* From **10b** 1.45 g (64 %) yellow platelets, mp 39 °C.– IR (NaCl): ν = 1700, 1640, 1540, 1335, 1270, 1205, 1150, 825, 730 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 5.48 (s, 2H, CH_2), 7.70 (mc, 4H, aromat.) 9.98 (s, 1H, CHO).– Anal. ($C_8H_7NO_4$).

*2-Nitrooxymethyl-benzonitrile (**12**)*

A solution of $AgNO_3$ (5.65 g, 33.2 mmol) in 20 ml acetonitrile was added dropwise to **9c** (6.0 g, 31 mmol) dissolved in 20 ml acetonitrile. The mixture was stirred at room temp. for 2 h. AgBr was separated, the filtrate evaporated and the residue thorougly washed with 3 × 100 ml water and extracted with 3 × 125 ml CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and evaporated. Crystallization on addition of ether and *n*-hexane. Recrystallization from *n*-hexane. 1.4 g (51 %) white crystals, mp 37 °C.– IR (NaCl): ν = 2200, 1640, 1540, 1410, 1330, 1270, 1215, 915, 850, 800 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 5.60 (s, 2H, CH_2), 7.62 (mc, 4H, aromat.).– Anal. ($C_8H_6N_2O_3$).

*Ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(4-nitrooxymethyl-phenyl)-pyridine-3,5-dicarboxylate (**3a**)*

11a (2.0 g, 11 mmol), methyl-β-aminocrotonate (1.27 g, 11 mmol) and ethyl acetoacetate (1.44 g, 11 mmol) were dissolved in acetonitrile (60 ml), protected from light and stirred under argon for 20 h at 35–38 °C. The mixture was evaporated and the orange residue purified by flash cc. 12 Fractions (300 ml) were gathered. Fractions 5–10 containing **3a** (R_f 0.5, acetone/petroleum ether 1:3) were combined and evaporated. Crystallization on addition of ether/petroleum ether, two recrystallization from methanol. 600 mg (13.9%) yellow crystals, mp 112 °C.– IR (KBr): ν = 3320, 2990, 1700, 1650, 1270 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.20 (t, *J* = 8 Hz, 3H, CH_2-*CH_3*), 2.29, 230 (2 × s, 2 × 3H, 2-CH , 6-CH_3), 3.61 (s, 3H, OCH_3), 4.09 (mc, 2H, *CH_2*-CH_3), 5.00 (s, 1H, 4-H). 5.36 (s, 2H CH_2ONO_2), 5.77 (s, 1H, NH), 7.25 (mc, 4H, aromat.).– MS (70 eV): *m/z* (%) = 390 (4.5) [M^+], 376 (5.5), 361 (1), 345 (4), 344 (1), 331 (2), 328 (5), 317 (1), 314 (8), 298 (12.5), 270 (13), 252 (41), 238 (94), 224 (100), 210 (31), 192 (10), 32 (15).– Anal. ($C_{19}H_{22}N_2O_7$).

*Ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrooxymethyl-phenyl)-pyridine-3,5-dicarboxylate (**3b**)*

Analog **3a** from **11b.** Fractions 4–9 out of 12 300 ml-fractions (same R_f as **3a**). 600 mg (13.9 %) yellow crystals, mp 90 °C.– IR (KBr): ν = 3310, 2980, 1695, 1650, 1270 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.20 (t, *J* = 8 Hz, 3H, CH_2-*CH_3*), 2.31, 2.32 (2 × s, 2 × 3H, 2-CH_3, 6-CH_3), 3.65 (s, 3H, OCH_3), 4.05 (mc, 2H, *CH_2*-CH_3), 4.98 (s, 1H, 4-H). 5.35 (s, 2H CH_2ONO_2), 5.70 (s, 1H, NH), 7.25 (mc, 4H, aromat.).– MS (70 eV): *m/z* (%) = 390 (11), 376 (16), 361 (2), 345 (11), 344 (2), 331 (3.5), 317 (2), 314 (22), 298 (43), 238 (85), 224 (100), 210 (20), 69 (13).– Anal ($C_{19}H_{22}N_2O_7$).

*2-Dimethylaminoethyl 5-cyano-1,4-dihydro-2,6-dimethyl-4-(3-nitrooxymethyl-phenyl)-pyridine-3-carboxylate (**3c**)*

Analog **3a** from **11b** (6.32 g, 35 mmol), β-aminocrotononitrile (2.86 g, 3 mmol) and 2-dimethylamino acetoacetate (5.2 g, 35 mmol) in 60 ml acetonitrile. Fractions 5–11 out of 14 300 ml-fractions (R_f 0,39). 900 mg (6.4%) yellow powder, mp 78 °C.– IR (KBr): ν = 3330, 2975, 2200, 1700, 1650, 1270 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.11 (s, 3H, 6-CH3), 2.17 (s, 6H, N(CH3)2), 2.37 (s, 3H, 2-CH_3), 2.43 (t, *J* = 6.5, 2H, CH2N), 4.04 (mc, 2H, O-CH_2), 4.75 (s, 1H, 4-H). 5.52 (s, 2H CH_2ONO_2), 6.26 (s, 1H, NH), 7.47 (dd, *J* = 8, 8 Hz, 1H, 5′-H), 7.64 (ddd, *J* = 8, 2, 1 Hz, 1H, 6′-H), 8.06 (mc, 1H, 4′-H), 8.10 (dd, *J* = 3, 2 Hz, 1H, 2′-H).– Anal. ($C_{20}H_{24}N_4O_5$).

*1,4-Dihydro-2,6-dimethyl-4-(3-nitrooxymethyl-phenyl)-pyridine-3,5-dicarbonitrile (**3d**)*

A solution of **11b** (2.15 g, 11.9 mmol) and β-aminocrotonnitrile (1.95 g, 23.8 mmol) in acetonitrile (60 ml) was stirred at 60 °C for 24 h. The mixture was evaporated. The oily residue crystallized on addition of *tert*-butylmethylether and was recrystallized twice from methanol. 200 mg (5.5 %) slightly coloured platelets, mp 149 °C.– IR (KBr): ν = 3350, 2960, 2210,

1650, 1270 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.05 (s, 6H, 2-CH_3, 6-CH_3), 4.55 (s, 1H, 4-H), 5.60 (s, 2H CH_2ONO_2), 6.15 (s, 1H, NH), 7.30 (mc, 4H, aromat.).– Anal. ($C_{16}H_{14}N_4O_3$).

Pharmacological methods

Inotropic effects: Inotropic activity was evaluated on electrically stimulated right trabecular muscles (1 Hz, 3 ms, 50 V), isolated from porcine hearts which were obtained from the local slaughter house. Transport of the hearts was performed in a carbogen (95 % O_2, 5 % CO_2) saturated with St. Thomas-Hospital cardioplegic solution (pH 7.8, 6 °C) of the following composition (in mmol/l): Na^+ 120.5, K^+ 16.0, Mg^{2+} 16.0, Ca^{2+} 1.2, Cl^- 160.7, HCO_3^- 10.1. Isolated trabecular muscles were incubated at 30 °C in an organ bath containing 10 ml of a Krebs-Henseleit solution of the following composition (in mmol/l): Na^+ 143.1, K^+ 5.9, Mg^{2+} 16.0, Ca^{2+} 1.6, Cl^- 126.0, HCO_3^- 25.0, $H_2PO_4^-$ 1.2, SO_4^- 1.2, glucose 5.1, being equilibrated with carbogen to attain a pH of 7.4. Contractile force was measured isometrically by means of a force displacement transducer and recorded after amplification (DMS DC Verstärker, Fleck, Mainz, Germany) on a thermorecorder (Hellige Servomed, Freiburg, Germany). Equilibration was allowed for 90 minutes at a resting tension of 0.5 g. Mean contractile force was 1.63 ± 0.18 mN (n = 36). All compounds studied were added in a cumulative manner at 30 min intervals. The time intervals were chosen such as to allow a steady state of drug action.

Vasodilator activity: Vasodilator activity was tested at a temperature of 37 °C on isolated right coronary arteries from the same hearts as described above. Proximal parts of the arteries were rapidly dissected from fresh hearts, cut into ring segments (3–4 mm), fixed between two stainless steel hooks as described by Toward[12] and incubated in a 10 ml organ bath containing Krebs-Henseleit solution (see above), which was equilibrated with carbogen to attain a pH of 7.4. Resting tension was 2 g. Vascular tone was measured isotonically using a strain gauge and was recorded after amplification (Fleck, Mainz, Germany) on a digital point printing recorder (Linsseis, Selb, Germany). After an equilibration period of 45 min, during which the tissues were frequently washed, the arterial rings were contracted in a depolarizing medium (60 mmol/l KCl) giving mean contractions of 36.27 ± 1.32 µJ [n = 41]. After stabilization each compound studied was added in a cumulative manner at 30 min intervals to allow steady state of drug action. Each individual concentration-effect curve was followed by the addition of 0.2 mmol/l papaverine for maximal dilatation.

Materials: Nitrendipine was a gift from Bayer AG, Leverkusen, Germany. All other chemicals including glyceryl trinitrate were purchased from Merck, Darmstadt, Germany. The dihydropyridines and GTN were dissolved in DMSO, which in concentrations up to 0.72 % in the organ bath did not affect myocardial and vascular contractility in control experiments. The experiments on the dihydropyridines were conducted under sodium light to avoid photodegradation. Solutions of test compounds in DMSO and DMSO/D_2O proved to be stable (dc) under these conditions.

Calculations: Results are expressed as percentage of initial contraction amplitude (ventricular trabeculae) or an initial KCl-induced contraction (coronary arteries). Drug concentrations for half-maximal depression of contractile force or vascular tension (EC50) were estimated from each individual concentration-effect-curve by LOGIT-transformation as indicated by Hafner et al.[13]. All data were analysed by standard statistical methods (mean value and standard error of mean).

References

[1] Part I: K. Sandrock, M. Feelisch, H. Bökens, J. Lehmann, C. Meese (Schwarz Pharma AG). Intern. Pat. Appl. (PCT) **1995**, WO 95/00477 [Chem. Abstr. **1995** *123*, P199401t].

[2] P. A. van Zwieten, M. Pfaffendorf, *J. Hypertension* **1993**, *11 (Supp. 1)*, 3–11.

[3] J. Günther, S. Dhein, R. Rösen, W. Klaus, U. Fricke, *Basic Res. Cardiol.* **1992**, *87*, 452–460.

[4] J. E. Mackenzie, J. R. Parratt, *Br. J. Pharmacol.* **1977**, *60*, 155–160.

[5] H. Yamaura, N. Miyata, K. Takahashi, K. Tsuchida, S. Otomo, *Eur. J. Pharmacol.* **1994**, *260*, 269–272.

[6] N. Miyata, H. Yamaura, M. Tanaka, K. Takahashi, K. Tsuchida, S. Otomo, *Eur. J. Pharmacol.* **1993**, *249*, 141–149.

[7] D. J. Triggle, D. A. Langs, R. A. Janis, *Medic. Res. Rev.* **1989**, *9*, 123–180.

[8] A. C. Gandolfi, M. Frigerio, S. Spinelli, O. Tofanetti, S. Tognella, E. P. 86110295.2, **1987** [Chem. Abstr. **1988** *108*, 21721z].

[9] T. Shibanuba, M. Iwanami, M. Fujimoto, T. Takenaka, M. Murakami, *Chem. Pharm. Bull.* **1980**, *28(9)*, 2609–2613.

[10] J. W. Baker, J. A. L. Brieux, D. G. Saunders, *J. Chem. Soc.* **1956**, 404–414.

[11] B. C. Bookser, T. C. Buice, *J. Am. Chem. Soc.* **1991**, *113*, 4208–4218.

[12] R. Towart, *Br. J. Pharmacol.* **1982**, *75*. 150P.

[13] D. Haffner, E. Heinen, E.A. Noack, *Arzneim. Forsch.* **1977**, *27*, 1871–1873.

Received: April 21, 1997 [FP207]