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Studies on condensed 1,4-dihydropyridine derivatives and their calcium modulatory activities

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Hexahydroquinoline and furoquinoline derivatives were synthesized and their calcium modulatory activity was investigated on isolated rat ileum and lamb carotid artery. In addition, the *in vitro* hepatic microsomal biotransformation of one hexahydroquinoline derivative was studied in rat microsomes.

1. Introduction

Calcium channel antagonists inhibit muscle contraction blocking the influx Ca^{2+} through calcium channels and were used as antianginal and antihypertensive drugs (Godfraind et al. 1986; Janis and Triggle 1983, 1984; Weiner 1988). Nifedipine, carrying 1,4-dihydropyridine (DHP) moiety in its structure, is the prototype of this group.

Many nifedipine-like compounds have been synthesized agonists or antagonists. Agonist and antagonist compounds have similar structural requirements and interact with different regions of the same receptor (Franckowiak et al. 1985; Lans and Triggle 1985; Rovnyak et al.1995). Although many dihydropyridine derivatives have structural properties similar to nifedipine, they are agonists. Many active compounds have also been obtained by the introduction of the 1,4-DHP moiety to condensed systems (Rose 1989, 1990a, 1990b, 1991; Şafak et al. 1993, 1995; Altaş et al. 1999). Active antagonists have an aromatic ring in the four position of the dihydropyridine ring, which tends both to restrict the aromatic ring to the DHP vertical plane and flatten the DHP ring. Biotransformation of nifedipine-like compounds results in many metabolites. It is well known that the 1,4-DHP derivatives are metabolized to their lactone derivatives by microsomal enzymes (Guangerich et al. 1988). The metabolites thus formed also exert calcium-modulating effects (Rose 1989).

In this study furoquinoline and hexahydroquinoline derivatives were investigated regarding their calcium modulatory activity and biotransformation.

2. Investigations, results and discussion

The hexahydroquinoline derivatives were prepared by a modification of the Hantzsch synthesis (Hantzsch 1882). In this reaction, first benzylidene derivatives are formed by the reaction of 4,4 (5,5)-dimethyl-l,3-cyclohexanedione and an appropriate aldehyde derivative. Then, benzylidene derivatives react in methyl(ethyl) aminocrotonate to give the corresponding hexahydroquinoline derivatives.

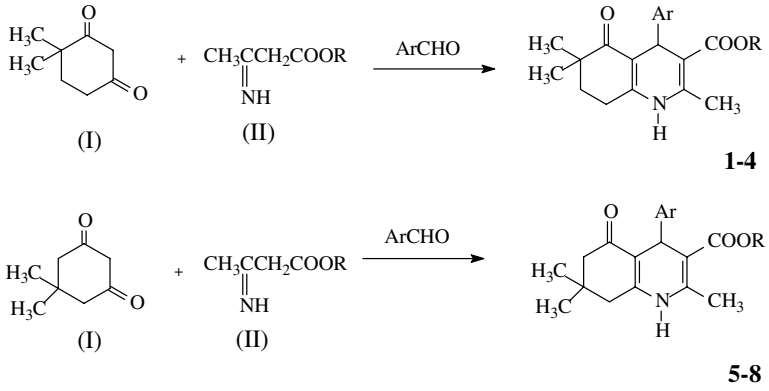
Furoquinoline compounds were obtained by reaction of an appropriate hexahydroquinoline derivative with pyridinium bromide perbromide (Rose 1989).

The structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, HMBC (Heteronuclear Long Range Correlation Spectrum), HSQC (Heteronuclear Single Quantum Coherence), mass spectra and elemental analyses. In the IR spectra, characteristic N−H and C=O stretching bands were seen. In the ^{1}H NMR spectra, 6-CH_3 protons of the hexahydroquinoline and 7-CH_3 protons of the furoquinoline ring were seen at 0.80–1.00 ppm as separate singlets. The chemical shifts of the aromatic, 2-methyl, methylene and methine protons of the compounds have expected values. The N−H signals were seen at 9–10 ppm. The ^{13}C NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of carbon atoms. The DEPT spectra of the compounds were in accordance with their structures. The MS of the compounds were recorded using the electron impact technique. Molecular ion peaks were seen in the MS of compounds. The base peak forms by cleavage of the aryl ring from the parent molecule. In further fragmentation, the ions are formed by the rupture of the cyclohexene ring and acylium ions are formed by the cleavage of the ester group. Aromatisation of the DHP ring to the pyridine analogue was also realised. These findings are accordance with the literature (Altaş et al. 1999).

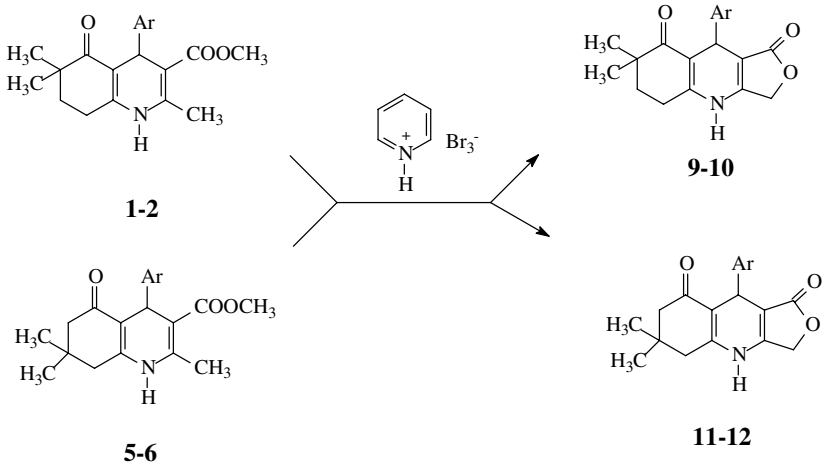
The X-ray diagram of compound **5** (Fig.) confirmed the proposed structure. X ray data show that an intermolecular hydrogen bond between the amine group and the carbonyl oxygene atom of the cyclohexanone ring of a neighboring molecule links the molecules have a graph-set motif of C (6) (Linden et al. 1998; Şimşek et al. 2000).

Finally, the elemental analysis results are also consistent with the postulated structures.

Scheme 1



Scheme 2



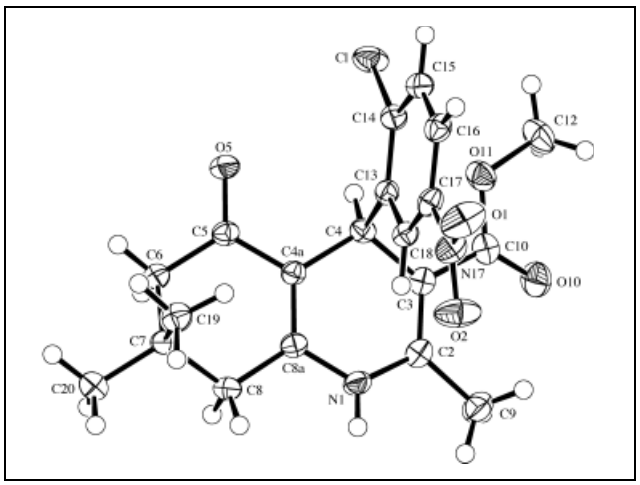


Fig.: X-ray diagramme of compound **5**

Calcium antagonistic activities of the compounds were determined by tests performed on isolated rat ileum and lamb carotid artery. In these studies, nicardipine was used as standard. The results are given in Tables 1 and 2.

On isolated rat ileum strips precontracted with barium chloride (4×10^{-3} mol/l), all compounds have been found less active than nicardipine in 10^{-6} M concentration. In 10^{-5} M concentration, compounds **4** and **6** were as active as nicardipine and compounds **2**, **3**, **7** and **8** have been found active but not like nicardipine. The results showed that ethyl esters are more active than methyl esters. The positions of the methyl groups in the hexahydroquinoline ring had no influence on activity. Also furoquinoline derivatives have not been found more active than hexahydroquinoline analogs.

In lamb carotid artery preparations precontracted with barium chloride (67 mmol/l), all compounds have been found more active than nicardipine in both 10^{-5} M and 10^{-6} M

Table 1: Relaxant effects of the compounds and nicardipine (10^{-6} mol/1) on isolated rat ileum precontracted with barium chloride (4×10^{-3} mol/l) (% ± SEM) (n = 7)

Compound	% Inhibition		EC_{50}
	10^{-6} M	10^{-5} M	
1	7.80 ± 2.33	35.31 ± 8.51	>10-^{5}
2	66.56 ± 5.12	98.38 ± 2.99	$5.80 \pm 3.32 \times 10^{-7}$
3	46.47 ± 8.29	89.74 ± 7.01	$3.74 \pm 1.97 \times 10^{-6}$
4	56.36 ± 5.99	100	$8.38 \pm 5.27 \times 10^{-7}$
5	15.74 ± 3.38	37.90 ± 6.70	$>10^{-5}$
6	41.07 ± 6.70	100	$2.17 \pm 1.66 \times 10^{-6}$
7	47.48 ± 8.47	96.04 ± 1.95	$1.68 \pm 0.75 \times 10^{-6}$
8	59.98 ± 4.27	91.94 ± 5.28	$6.38 \pm 3.42 \times 10^{-7}$
9	16.67± 3.23	10.20 ± 3.71	$>10^{-5}$
10	0	11.37 ± 0.91	$>10^{-5}$
11	11.88 ± 1.40	10.67 ± 2.73	$>10^{-5}$
12	13.70 ± 1.89	12.41 ± 1.24	$>10^{-5}$
Nicardipine	96.70 ± 8.50	100	$4.53 \pm 3.25 \times 10^{-9}$

Table 2: Relaxant effects of the compounds and nicardipine (10^{-6} mol/l) on lamb carotid artery precontracted with barium chloride (67 mmol/l) (% ± SEM) (n = 7)

Compound	% Inhibition	
	10^{-6} M	10^{-5} M
2	9.91 ± 1.42	21.70 ± 6.14
3	11.88 ± 2.42	25.61 ± 5.83
4	20.41 ± 3.35	37.40 ± 8.68
6	7.06 ± 2.94	14.15 ± 3.50
7	15.42 ± 2.11	38.42 ± 10.75
8	15.53 ± 1.89	32.97 ± 9.03
Nicardipine	7.32 ± 2.30	14.34 ± 3.13

EC_{50} values were found as $>10^{-5}$ for all compounds

concentrations except compound **6**. In 10^{-6} M concentration, compound **4** was the most active. The results showed that ethyl esters are more active than methyl esters at both concentrations. These findings are in accordance with the results which are obtained on the rat ileum. In addition, the position of methyl groups in hexahydroquinoline had no influence on activity. Results obtained are in accordance with the structure-activity relationships of these derivatives.

Compound **5** was metabolized to its lactone derivative **11**. This was verified by HPLC and is in accordance with the biotransformation of similar structures.

3. Experimental

3.1. Chemistry

All chemicals used in this study were purchased from Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland). Pyridinium bromide perbromide was prepared according to the literature (Fieser and Fieser 1967).

Melting point: Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA); the values are uncorrected. UV spectra: Shimadzu UV-160A UV-Visible Spectrophotometer (Shimadzu Co., Kyoto, Japan). IR spectra: Perkin Elmer FT-IR Spectrophotometer 1720 X (Beaconsfield, UK) (KBr disc) (γ, cm^{-1}). ^{1}H NMR spectra: Bruker DPX-400 MHz Digital FT NMR and H^1 AMX 600 MHz FT NMR Spectrophotometer (Karlsruhe, Germany) (DMSO-d_6; tetramethylsilane as internal standard). 1D and 2D-NMR spectra Bruker AMK-600 MHz FT NMR. ^{13}C NMR Spectra: ^{13}C AMX 150 MHz FT NMR Spectrophotometer. Chemical shift values are given as ppm. MS: Hewlett Packard Series II Plus 5890 GAS Cromatograph-Hewlett Packard 5972 Series Mass Selective Detector (Philadelphia, USA). TLC was run on precoated (0.2 mm) silica gel Merck 254 + 366 (E. Merck, Darmstadt, Germany) and short wave UV light (254 nm) was used to detect the UV absorbing spots. Elemental analysis was carried out an a Leco 932 CHNS-O Elemental Analyzer (Philadelphia, USA) (TUBITAK, Ankara, Turkey). The elemental analysis results were within 0.4% of theoretical values. All measurements were done on Nonius Kappa CCD difractometer using graphite-monochromatised M_0 Kα radiation (λ = 0.71073 Å) for X-ray study.

3.1.1. Synthesis of methyl(ethyl) 4-aryl-2,6,6(and 2,7,7)-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylates **1–8**

Equimolar amounts of 4,4(5,5)-dimethyl-1,3-cyclohexanedione (I) methyl-(ethyl) aminocrotonate (II) and aryl aldehyde were refluxed with × 20 mL methanol for 4 h. The forming precipitate was filtered and crystallized from alcohol.

3.1.1.1. Methyl 4-(2-chloro-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**1**)

Yield 78%, M.p. 205 °C. IR (cm^{-1}) 3286, 1703, 1608. ^{1}H NMR (ppm) 0.80 (3 H; s; 6-CH_3), 0.95 (3 H; s; 6-CH_3), 1.65 (2 H; t; H-7), 2.30 (3 H; s; 2-CH_3), 2.45 (2 H; t; H-8), 3.60 (3 H; s; $COOCH_3$), 5.20 (1 H; s; H-4), 7.40–8.10 (3 H; m; Ar−H), 9.15 (1 H; s; NH). ^{13}C NMR (ppm) 203, 170, 152, 149, 148, 148, 141, 131, 127, 123, 111, 104, 51, 41, 50, 50, 38, 25, 25, 20. Mass (m/e) 404, 387, 369, 345, 303, 272, 248 (%100), 232, 218, 204, 190, 165, 152, 139, 91, 77, 59, 41.
$C_{20}H_{21}ClN_2O_5$ (404.8)

3.1.1.2. Methyl 4-(2-chloro-6-fluorophenyl)-2,6,6-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**2**)

Yield 71%, M.p. 150 °C. IR (cm^{-1}) 3286, 1698, 1602. ^{1}H NMR (ppm) 0.80 (3 H; s; 6-CH_3), 0.95 (3 H; s; 6-CH_3), 1.65 (2 H; t; H-7), 2.30 (3 H; s; 2-CH_3), 2.45 (2 H; t; H-8), 3.50 (3 H; s; $COOCH_3$), 5.30 (1 H; s; H-4), 6.70–7.30 (3 H; m; Ar−H) 9.10 (1 H; s; NH). Mass (m/e) 377, 362, 342, 282, 248 (%100), 232, 204, 190, 157, 91, 77, 59, 41.
$C_{20}H_{21}ClFNO_3$ (377.8)

3.1.1.3. Ethyl 4-(2-chloro-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3**)

Yield 81%, M.p. 197 C. IR (cm^{-1}) 3287, 1694, 1611. ^{1}H NMR (ppm) 0.80 (3 H; s; 6-CH_3), 0.95 (3 H; s; 6-CH_3), 1.15 (3 H; t; CH_2CH_3), 1.70 (2 H; t; H-7), 2.25 (3 H; s; 2-CH_3), 2.45 (2 H; t; H-8), 3.95 (2 H; q; CH_2CH_3), 5.15 (1 H; s; H-4), 7.30–8.20 (3 H; m; Ar−H), 9.20 (1 H; s; NH). Mass (m/e) 418, 401, 381, 353, 262 (%100), 234, 190, 91, 77, 63, 41.
$C_{21}H_{23}ClN_2O_5$ (418.9)

3.1.1.4. Ethyl 4-(2-chloro-6-fluorophenyl)-2,6,6-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4**)

Yield 77%, M.p. 208 °C. IR (cm^{-1}) 3315, 1697, 1602. ^{1}H NMR (ppm) 0.75 (3 H; s; 6-CH_3), 0.95 (3 H; s; 6-CH_3), 1.10 (3 H; t; CH_2CH_3), 1.70 (2 H; t; H-7), 2.30 (3 H; s; 2-CH3), 2.45 (2 H; t; H-8), 3.95 (2 H; q; CH_2CH_3), 5.35 (1 H; s; H-4), 6.70–7.30 (3 H; m; Ar−H), 9.20 (1 H; s; NH). ^{13}C NMR (ppm) 201, 167, 149, 145, 141, 135, 131, 127, 127, 114, 114, 108, 77, 60, 40, 34, 32, 24, 22, 19, 14. Mass (m/e) 391, 356, 318, 262 (%100), 234, 199, 157, 105, 77, 59, 41.
$C_{20}H_{23}ClFNO_3$ (391.9)

3.1.1.5. Methyl 4-(2-chloro-5-nitrophenyl)-2,7,7-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5**)

Yield 75%, M.p. 240 °C. IR (cm^{-1}) 3286, 1702, 1631. ^{1}H NMR (ppm) 0.80 (3 H; s; 7-CH_3), 1.05 (3 H; s; 7-CH_3), 2.10 (2 H; s; H-6), 2.30 (3 H; s;

2-CH_3), 2.50 (2 H; s; H-8), 3.40 (3 H; s; $COOCH_3$), 5.30 (1 H; s; H-4), 7.35–8.15 (3 H; m; Ar–H), 9.20 (1 H; s; NH). ^{13}C NMR (ppm) 192, 164, 147, 143, 143, 136, 127, 123, 118, 106, 100, 47, 46, 45, 36, 32, 28, 25, 23, 15. DEPT-135 (ppm) 15 (CH_3), 23 (CH_3), 25 (CH_3), 32 (CH), 36 (CH_2), 46 (CH_2), 47 (CH_3), 118 (CH), 123 (CH), 127 (CH). Mass (m/e) 404, 387, 369, 345, 249 (%100), 224, 192, 166, 149, 132, 105, 77, 43.
$C_{20}H_{21}ClN_2O_5$ (404.8)

3.1.1.6. Methyl 4-(2-chloro-6-fluorophenyl)-2,7,7-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**6**)

Yield 70%, M.p. 178 °C. IR (cm^{-1}) 3290, 1702, 1608. ^{1}H NMR (ppm) 0.80 (3 H; s; 6-CH_3), 0.95 (3 H; s; 6-CH_3), 2.00 (2 H; s; H-6), 2.30 (3 H; s; 2-CH_3), 2.50 (2 H; s; H-8), 3.40 (3 H; s; $COOCH_3$), 5.30 (1 H; s; H-4), 6.50–7.60 (3 H; m; Ar–H), 9.20 (1 H; s; NH). Mass (m/e) 377, 342, 275, 261, 248 (%100), 232, 205, 149, 77, 63, 43.
$C_{20}H_{21}ClFNO_3$ (377.8)

3.1.1.7. Ethyl 4-(2-chloro-5-nitrophenyl)-2,7,7-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**7**)

Yield 83%, M.p. 197 °C. IR (cm^{-1}) 3292, 1703, 1609. ^{1}H NMR (ppm) 0.75 (3 H; s; 7-CH_3), 0.90 (3 H; s; 7-CH_3), 1.20 (3 H; t; CH_2CH_3), 1.95 (2 H; s; H-6), 2.25 (3 H; s; 2-CH_3), 2.45 (2 H; s; H-8), 3.85 (2 H; q; CH_2CH_3), 5.30 (1 H; s; H-4), 7.40–8.20 (3 H; m; Ar–H), 9.20 (1 H; s; NH). Mass (m/e) 418, 381, 351, 252 (%100), 224, 196, 150, 75, 45, 31.
$C_{21}H_{23}ClN_2O_5$ (418.9)

3.1.1.9. Ethyl 4-(2-chloro-6-fluorophenyl)-2,7,7-trimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**8**)

Yield 66%, M.p. 205 °C. IR (cm^{-1}) 3285, 1692, 1614. ^{1}H NMR (ppm) 0.80 (3 H; s; 7-CH_3), 0.95 (3 H; s; 7-CH_3), 1.10 (3 H t; CH_2CH_3), 1.90 (2 H; s; H-6), 2.25 (3 H; s; 2-CH_3), 2.45 (2 H; s; H-8), 3.80 (2 H; q; CH_2CH_3), 5.30 (1 H; s; H-4), 6.55–7.50 (3 H; m; Ar–H), 9.20 (1 H; s; NH). Mass (m/e) 391, 356, 318, 262 (%100), 234, 91, 77, 63, 42.
$C_{20}H_{23}ClFNO_3$ (391.9)

3.1.2. 9-Aryl-7,7-dimethyl-1,3,4,5,6,7,8.9-octahydrofuro[3,4-b]quinoline-1,8-diones **9**–**12**

2,6,6(2,7,7)-Trimethyl-3-carbmethoxy-4-aryl-5-oxo-l,4,5,6,7,8-hexahydroquinoline derivatives **1**, **2**, **5**, **6** (0.5 mmol) and 0.6 mmol pyridinium bromide perbromide in chloroform were mixed and refluxed for 4 h. The forming oily compound was solidified with petroleum ether. All furoquinoline derivatives were crystallized from alcohol.

3.1.2.1. 9-(2-Chloro-5-nitrophenyl)-7,7-dimethyl-1,3,4,5,6,7,8,9-octahydrofuro[3,4-b]quinoline-1,8-dione (**9**)

Yield 59%, M.p. 286 °C. IR (cm^{-1}) 3203, 1722, 1670. ^{1}H NMR (ppm) 1.05 (3 H; s; 7-CH_3), 1.10 (3 H; s; 7-CH_3), 1.90 (2 H; t; 5-CH_2), 2.60 (2 H; t; 6-CH_2), 5.00 (2 H; s; 3-CH_2), 5.30 (1 H; s; H-9), 7.70–8.10 (3 H; m; Ar–H), 10.30 (1 H; s; NH). ^{13}C NMR (ppm) 202, 172, 152, 147, 144, 140, 130, 125, 122, 110, 102, 78, 49, 49, 49, 48, 34, 24, 24. Mass (m/e) 388, 371, 353, 335, 305, 232 (%100), 216, 176, 91, 75, 55, 41.
$C_{19}H_{17}ClN_2O_5$ (388.8)

3.1.2.2. 9-(2-Chloro-6-fluorophenyl)-7,7-dimethyl-1,3,4,5,6,7,8.9-octahydrofuro[3,4-b]quinoline-1,8-dione (**10**)

Yield 66%, M.p. 275 °C. IR (cm^{-1}) 3267, 1746,1682. ^{1}H NMR (ppm) 0.80 (3 H; s; 7-CH_3), 0.95 (3 H; s; 7-CH_3), 1.80 (2 H; t; 5-CH_2), 2.50 (2 H; t; 6-CH_2), 4.90 (2 H; s; 3-CH_2), 5.30 (1 H; s; H-9), 6.80–7.30 (3 H; m; Ar–H), 10.10 (1 H; s; NH). Mass (m/e) 361, 346, 326, 282, 232 (%100), 216, 176, 157, 91, 77, 63, 41.
$C_{19}H_{17}ClFNO_3$ (361.8)

3.1.2.3. 9-(2-Chloro-5-nitrophenyl1-6,6-dimethyl-1,3,4,5,6,7,8.9-octahydrofuro[3,4-b]quinoline-1,8-dione (**11**)

Yield 70%, M.p. 156 °C. IR (cm^{-1}) 3203, 1722, 1670. ^{1}H NMR (ppm) 0.80 (3 H; s; 6-CH_3), 0.95 (3 H; s; 6-CH_3), 2.10 (2 H; s; 5-CH_2), 2.50 (2 H; s; 7-CH_2), 4.80 (2 H; s; 3-CH_2), 5.30 (1 H; s; H-9), 7.30–9.25 (3 H; m; Ar–H), 10.30 (1 H; s; NH). Mass (m/e) 388, 371, 351 (%100), 335, 321, 305, 289, 232, 176, 82, 63, 41.
$C_{19}H_{17}ClN_2O_5$ (388.8)

3.1.2.4. 9-(2-Chloro-6-fluorophenyl)-6,6-dimethyl-1,3,4,5,6,7,8.9-octahydrofuro[3,4-b]quinoline-1,8-dione (**12**)

Yield 56%, M.p. 213 °C. IR (cm^{-1}) 3155, 1758, 1692. ^{1}H NMR (ppm) 0.80 (3 H; s; 6-CH_3), 1.10 (3 H; s; 6-CH_3), 2.10 (2 H; s; 5-CH_2), 2.40 (2 H; s; 7-CH_2), 4.80 (2 H; s; 3-CH_2), 5.30 (1 H; s; H-9), 6.60–7.65 (3 H; m; Ar–H), 10. (1 H; s; NH). Mass (m/e) 361, 326, 302, 282, 261 (%100), 205, 176, 157, 82, 63, 41.
$C_{19}H_{17}ClFNO_3$ (361.8)

3.2. Pharmacology

The calcium antagonistic activities of the compounds were determined by tests performed on isolated rat ileum and lamb carotid artery. All procedures involving animals and their care were conducted in conformity with international laws and policies.

3.2.1. Studies on isolated rat ileum (Kazda et al. 1980)

Albino rats of either sex (weighing 150–200 g) were used. Six experimental animals were used per experiment. They were supplied from the Laboratory Animal Production Center in the Department of Pharmacology, School of Medicine, Osmangazi University, Eskişehir (Turkey). Animals used in the test were fasted overnight. After the animals were sacrificied by cervical dislocation, the ileum (10–15 cm terminal portion) was immediately removed, and a 5–8 cm segment proximal to the ileocaecal junction was discarded. Segments 1.5–2 cm long were mounted vertically in a 10 ml organ bath containing Tyrode solution of the following composition (mmol/1): NaCl: 136.87; KC1: 2.68; $CaCl_2$:1.80; $MgSO_4$ 0.81; NaH_2PO_4: 4.16; $NaHCO_3$: 11.9; glucose: 5.55. The bath contents were maintained at 37 °C and aerated by 95% O_2 and 5% $C0_2$. A tension of 2 g was applied and isometric recording was done by an isometric transducer (FDT_{10}-A) MAY TDA95 Transducer Data Acquisition System (MAY, Commat, Ankara, Turkey). The preparations were allowed to equilibrate for 60 min with regular washes every 15 min in order to check for antagonistic effects, contractions were induced with barium chloride (3×10^{-3} mol/l, bath concentration). After washing out, this process was repeated until the amplitude of the contraction became constant. Investigations of the substances were performed using the single dose technique. Barium chloride contractions were induced after addition of the test substances dissolved in dimethylsulphoxide at different concentrations (10^{-6} mol/l) and 5 min exposure time. Only one compound was tested in each preparation. In order to check for calcium antagonistic effects, contractions of the isolated ileum were induced with barium chloride.

3.2.2. Studies on lamb carotid artery (Altaş et al. 1999)

Sheep (*Ovis aries*) carotid artery preparations were obtained from the local slaugterhouse. Rings (3 mm) were suspended in organ baths of 10 mL capacity which contained Tyrode solution of the following composition (mmol/1): NaCl: 136.87; KC1: 2.68; $CaCl_2$: 1.80; $MgSO_4$: 0.81; NaH_2PO_4: 4.16; $NaHCO_3$: 11.9; glucose: 5.55. The bath contents were maintained at 37 °C and aerated by 95% O_2 and 5% $C0_2$. in a gas of ±95% O_2 and 5% CO_2 and a tension of 2 g was applied. The preparations were allowed to equilibrate for 60 min with regular washes every 15 min in order to check for antagonistic effects, contractions were induced with 67 mmol/l potassium chloride. After washing out, this process was repeated until the amplitude of the contraction became constant. Investigations of the substances were performed using the single dose technique. Potassium chloride contractions were induced after addition of the test substance and 10 min exposure time. During administration of the individual substances, the preparation was washed until the initial situation had been reestablished and the potassium chloride contractions were induced. The isometric contractions were recorded by an isometric transducer (FDT_{10}-A) May TDA95 Transducer Data Acquisition System (May, Commat, Ankara, Turkey).

3.3. Biotransformation studies

The *in vitro* microsomal metabolism studies were performed in rat microsomes which were prepared according to the calcium chloride precipitation technique. Then *in vitro* microsomal oxidation was used to convert the hexahydroquinoline derivative **5** to the furoquinoline derivative **11**. This metabolism was also proven by HPLC data.

3.3.1. Microsomal preparations (Ülgen 1992)

The animals were sacrified by cervical dislocation and the liver was removed, cut into small pieces and homogenised with sucrose solution (0.25 mol/L) to make a 25% v/v homogenate. The homogenate was frst centrifuged at 10500 rpm at 4 °C for 30 min to remove cell debris, intact cells, nuclei and mitochondria. The supernatant containing the microsomal and soluble cell fractions was carefully decanted and its volume was measured. Calcium chloride solution (80 mmol/L) was added to the supernatant (l ml for 9 mL supernatant) and the mixture was then centrifuged at 15500 rpm for 15 min. For the washing step, the pellet containing microsomes was resuspended in potassium chloride solution (0.15 mol/L), in a volume equal to first supernatant. Finally, the microsomes were washed by centrifuging this homogenate at 15,500 rpm for 15 min. The pellet (washed microsomes) was resuspended in phosphate buffer (0.2 mol/L, pH 7.4) and the final suspension contained l g original liver per l mL. This was used

freshly or stored at −80 °C until required. The production of NADPH was effected by incubation of cofactors. Substrates and microsomal preparations were incubated in a water bath for 30 min. Metabolic extracts were injected into a reversed HPLC system.

3.3.2. HPLC conditions

The characteristics of the HPLC system were as follows: HPLC pump (Gilson 302, Villiers le Bel, France), injection valve (Rheodyne 7215, Rohnert Park, CA, USA), UV detector (Spectromonitor LDC Model 1204A UV Spectrophotometer, UK), HPLC integrator (C-R8A Chromatopac, Shimadzu, Kyoto, Japan), reverse phase column (Waters, m-Bondapak C18 column, USA), Mobile phase: 50 : 50 (acetonitrile: waterv/v), flow velocity: 1 ml/min, maximum absorption: 254 nm.

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ERRATUM

Unfortunately the name of one author of the contribution "Increased dissolution and physical stability of micronized nifedipine particles encapsulated with a biocompatible polymer and surfactants in a wet ball milling process", Pharmazie 61 (2006), 595–603 was misspelled. The name of the author is A. S. ZAHR instead of A. S. ZAHN.

The correct title is

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Increased dissolution and physical stability of micronized nifedipine particles encapsulated with a biocompatible polymer and surfactants in a wet ball milling process

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