Turk J Chem
24 (2000) , 255 – 259.

Synthesis of a New Dihydropyridine Derivative Containing a Known Vasodilator Khellin

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Received 02.06.1999

The synthesis and spasmolytic activity of a novel dihydropyridine derivative which contains a vasodilator furanochromone khellin at the 4th position of the dihydropyridine ring via the Hantzsch method from khellin-2-carboxaldehyde is described.

Introduction

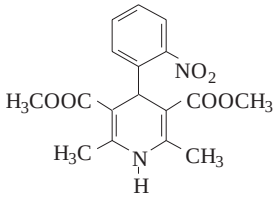
Dihydropyridine calcium antagonists, also called calcium channel inhibitors or calcium entry-blocking agents, are a known class in a wider class of calcium antagonists, which are among the most commonly used drugs for patients with cardiovascular diseases[1] (Scheme 1). The predominant pharmacological effects of calcium antagonists are coronary, peripheral and cerebral vasodilation[2], negative inotropic effect[3,4] and inhibition of excitation of sinoatrial and atrioventricular nodes[5]. These effects explain their remarkable therapeutic value in angina pectoris, hypertension, posthemorrhagic cerebral vasospasm and supraventricular tachycardia(5). Other effects have also been reported such as inhibition of platelet aggregation[6−9], relief of migraine[10], protection of tissues from calcium damage[11], tromboxane A_2 synthase inhibition[1] and antiperoxidant activity[12].

Khellin **(I)** is a vasodilator furanochromone derivative, isolated from seeds of *Amni visnage*, a fruit that has been used in folk medicine for its antispasmodic properties and in the treatment of angina pectoris[13]. We attempted to synthesize a new dihydropyridine derivative by introducing furanochromone ring system at the 4th position of the dihydropyridine nucleus and to evaluate its antiplatelet and calcium antagonist activities. The new compound is represented in Formula 1.

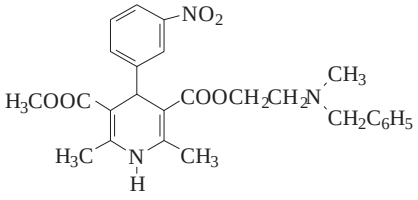
Experimental

Melting points were determined with an Electrothermal 9100 melting point apparatus and were uncorrected. IR spectra were recorded on a Jasco FT/IR 420 spectrophotometer as potassium bromide disks. ^{1}H NMR spectra were measured on a Bruker GmbH DPX-400, 400MHz instrument using TMS internal standard and $CDCl_3$. All chemical shifts were reported as δ (ppm) and coupling constants (J) are given in Hz. MS

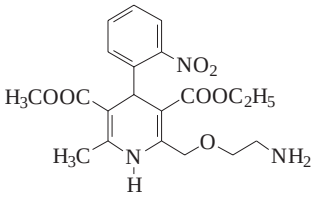
analysis was carried out on a VG Platform II LC-MS spectrometer (70eV) with EI methods. Elementary analysis was performed on a Leco 932 CHNS-O analyzer. All values of C, H, N were within ±0.4% of the calculated data. Column chromatography was performed using silica gel (Merck Art. 9385). All the chemical reagents used in the synthesis were purchased from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, WI,USA). Khellin-2-carboxaldehyde was synthesized from khellin (5,8-dimethoxy-2-methyl-4′,5′-furo-6,7-chromone) according to the method in reference 14. In this method, condensation of khellin **(2)** with 4-nitrosodimethylaniline**(1)** in the presence of sodium ethoxide, was followed by hydrolysis of the obtained nitrone **I** with sulphuric acid, which afforded khellin-2-carboxaldehyde **II**. Compound **III** was prepared using the Hantzsch reaction[15] (Scheme 2). In ^{1}H NMR spectra, the characteristic protons belonging the furanochromone and dihydropyridine moiety were observed at various δ ppm values. All spectral data were in accordance with assumed structures.



Nifedipine

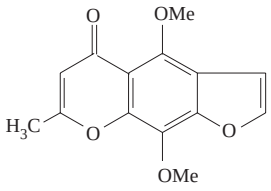


Nicardipine

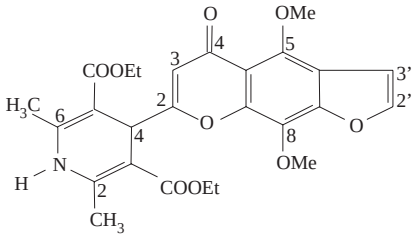


Amlodipine

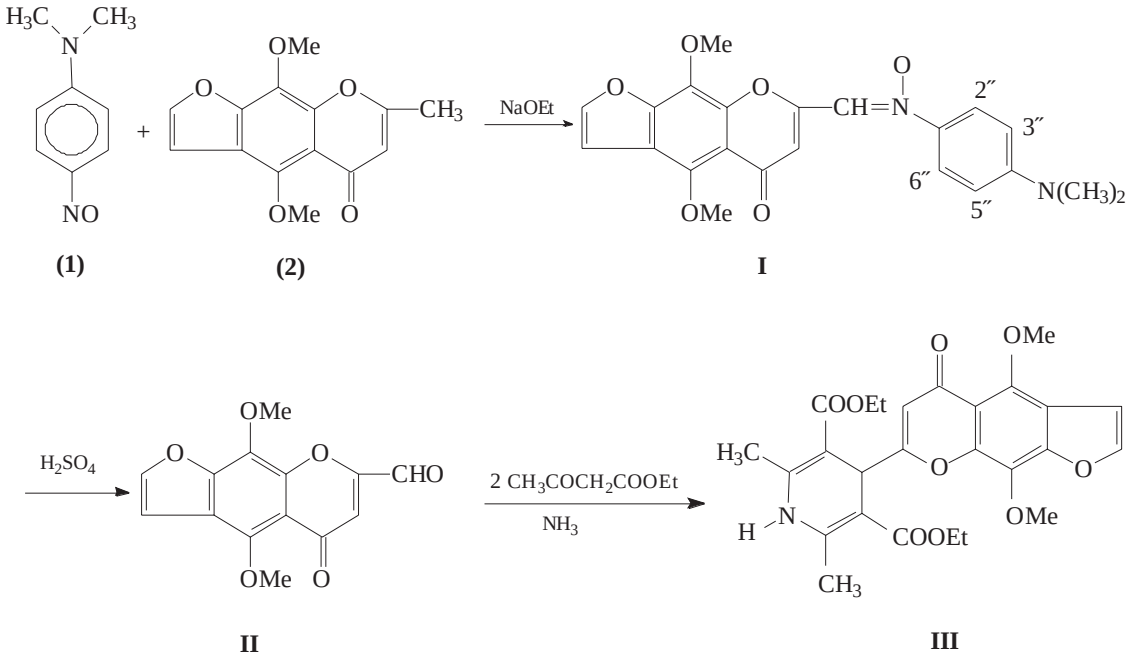
Scheme 1 Representative Calcium Antagonists of Dihydropyridine Class



(I)



Formula 1



Scheme 2 Synthesis of Compound **III**

5,8-dimethoxy-4′,5′-furo-6,7-chromone-2-carboxaldehyde (II)

A solution of sodium ethoxide [(prepared 44mg (2mmol) of Na and 15ml of ethanol)] was added dropwise to a cold (-5 to 0°C) solution of the khellin (0.52g, 2mmol) and 4-nitroso-N,N-dimethylaniline (0.9g, 6mmol) in a minimum amount of absolute ethanol and the mixture was set aside at room temperature for 10 min. The red precipitate was collected, washed with ethanol and then with ether. The product was purified by column chromatography on silica gel eluting with chloroform. 0.17g (yield; 20%) **I** was obtained, m.p: 210°C. IR cm^{-1}:1603 (CO). ^{1}H NMR ($CDCl_3$) (δ ppm): 3.05 (s, 6H, $N(CH_3)_2$), 4.08 (s, 3H, 5-OMe), 4.21 (s, 3H, 8-OMe), 6.68 (d, 2H, J_o=8.85, H-3″,5″), 7.03 (s, 1H, H-3), 7.63 (s, 1H, H-3′), 7.71 (d, 2H, J_o=8.89, H-2″,6″), 8.02(s, 1H, -HC=N-O), 8.14 (s, 1H, H-2′). Mass, EI 70eV: *m/z* (%): 408($M^{+\cdot}$,42.0), 392.2(33.0), 377.1(50.0), 196.2(7.6), 135.0(100.0), 121.2(19.9), 76.9(34.9).Anal. (C,H,N) for $C_{22}H_{20}N_2O_6$.

Calcd.	C: 64.70	H: 4.90	N: 6.86
Found	C: 64.53	H: 4.96	N: 6.70

A mixture of nitrone **I** (0.17g, 0.4mmol) and 5M H_2SO_4 (1.7ml) was stirred at room temperature for 15 min. After dilution with water (5ml), the precipitate was collected and purified by column chromatography using Hexane:EtOAc (1:1, v/v) as eluent. 0.076g (67%) **II** was obtained. m.p=179°C. (Lit.16 m.p=182-184°C).

Diethyl-1,4-dihydro-2,6-dimethyl-4-[5,8-dimethoxy-4′,5′-furo-6,7-chromone-2-yl]-3,5-pyridine dicarboxylate (III)

0.065g (0.024mmol) IIa, 0.48mmol ethylacetoacetate and 0.1ml ammonia (25%, v/v) were refluxed in 3ml isopropanol for 12 h. The solvent was removed and the residue was purified by column chromatography using Hexane:EtOAc (1:1, v/v), as eluent. 0.035g (30%) product was obtained. m.p: 244°C. IR cm^{-1}:1616 (CO), 1700 (COOEt). ^{1}H NMR ($CDCl_3$)(δ ppm): 1.29(t, 6H, $-CH_2CH_3$), 2.39 (s, 6H, DHP 2,6-CH_3), 4.03

(s, 3H, 5-OMe), 4.10 (s, 3H, 8-OMe), 4.21 (q, 4H, CH_2CH_3), 5.19 (s, 1H, DHP H-4), 6.05 (s, 1H, NH), 6.31 (s, 1H, H-3), 6.99 (d, 1H, $J_{2,3}$=1.76, H-3′), 7.59 (d, 1H, $J_{3,2}$=1.79, H-2′), Mass, EI 70eV: m/z (%): 498 (M+1, 24.11), 497($M^{+\cdot}$, 39.05), 482(3.81), 468(1.05), 452(3.6), 252(23.4), 224(2.1), 196(3.7), 84.8(64.5), 82.8(100), Anal. (C,H,N) for $C_{26}H_{27}NO_9.0.6H_2O$

Calcd.	C: 61.44	H: 5.55	N: 2.75
Found	C: 61.49	H: 5.84	N: 2.77

Pharmacology

Albino rats of either sex, weighing 200-220g were used in the present study (experiments were approved by Osmangazi University, School of Medicine, Animal Use and Care Committee). The animals were fasted overnight. After sacrifice by cervical dislocation, the ileum (10-15 cm terminal portion) was immediately removed. Segments 1.5-2 cm long were mounted vertically in a 10 ml organ bath containing Tyrode solution of the following composition (mM): NaCl:136.87; KCl:2.68; $CaCl_2$:1.80; $MgSO_4$:0.81; NaH_2PO_4:4.16; $NaHCO_3$:11.9; Glucose:11.1. The bath contents were maintained at 37°C and aerated by 95%O_2 and 5%CO_2. A tension of 2g was applied and isometric recording was done by using an isometric transducer (FDT10-A) MAYTDA95 Transducer Data Acquisition System (produced in 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 ($4x10^{-3}$ mol/l, bath concentration). After thorough washing, this process was repeated until the amplitude of the contraction became constant. Investigations of the substance to be tested were performed using the single dose technique in which the barium chloride contractions were induced after addition of the tested substance dissolved in dimethylsulphoxide at different concentrations (10^{-6}, 10^{-5}, 10^{-4} mol/l) and 5 min exposure time. The response of $BaCl_2$ was recorded after the incubation of 0.1ml DMSO. The response was expressed as % inhibition of $BaCl_2$ contractions.

Results and Discussion

Bioassay preparations such as isolated right (chronotropy) and left (inotropy) atria of guinea pig and rabbit portal vein, aortic strips of the rabbit, isolated papillary muscle of the guinea pig[17], guinea pig tenia coli in K^+-depolarizing Tyrode solution[18], isolated guinea pig ileum (Ba^{++} stimulation) [17] radioligand binding method[19] and hypotensive activity[20] are appropriate for pharmacological screening tests of calcium antagonistic activity. In the present study, $BaCl_2$-stimulated rat ileum was used. The dihydropyridines exhibit organ-specific activities at dihydropyridine receptors (DPR) [21]. For example, BAYK 5552 and nifedipine showed their calcium antagonistic activities at low concentration on portal vein and aortic strip whereas their activities on ileum right and left atrium and papillary muscle were observed at high concentration[17] .

In addition, it is known that the DHP receptors exhibit stereospecifity: the optical antipodes of asymmetrical dihydropyridines often possess not only differing receptor affinities, but sometimes also generate opposing effects[22]. These are the first screening tests for the calcium antagonistic activity of this compound. The compound exhibited no activity on rat ileum at 10^{-4} M concentration.

Further investigation such as the tests on isolated artery and vein preparation and radioligand binding assay are necessary to clarify this previous observation. Moreover, the stabilities of the tested compound in solution towards the action of light is worthy of note.

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