Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (6): 922-6 (2009)

Short Communication

Received: July 27, 2009
Accepted: July 31, 2009

7-Substituted Hexahydroquinoline Derivatives and their Calcium Channel Modulator Effects

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SUMMARY. **In this study, calcium antagonistic activity of sixteen hexahydroquinoline derivatives have been elucidated in rat ileum and rat thorasic aorta. In the studies on isolated rat ileum, it has been observed that all compounds showed meaningfull activity. In rat thorasic aorta studies, compound 3a has been found more active than nicardipine.**

INTRODUCTION

The fact that calcium ions regulate enzymatic reactions, activation of excitable cells and contraction of muscle, has well-known for years [1]. Calcium channel antagonists inhibit muscle contraction blocking the influx Ca^{2+} through calcium channels and were used as antianginal and antihypertensive drugs [2-5]. Although there are many type of compounds acting on calcium channels, the discovery of 1,4-dihydropyridines (DHPs) has been key stone and advantage in this area. The introduction of 1,4-DHPs with potent calcium channel antagonist activity led to new dimensions in cardiovascular therapy. Nifedipine, carrying 1,4-dihydropyridine (DHP) moiety in its structure, is the prototype drug of this group .

Many nifedipine-like compounds have been synthesised by making various modifications on nifedipine molecule. These modifications give agonist or antagonist compounds. Agonist and antagonist compounds have similar structural requirements and interact with different region of the same receptor [6]. Some changes yielded that the 1,4-DHP ring give the compounds exhibiting pharmacological actions opposite to those of nifedipine. Active compounds have also been

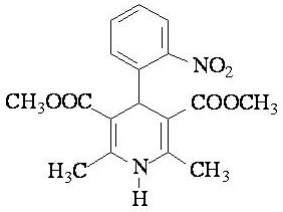

Figure 1. Nifedipine.

obtained by the introduction of the 1,4-DHP moiety to condensed systems such as hexahydroquinoline (HHQ), acridine, and furoquinoline [7-9]. Thus the carbonyl group of ester at the fifth position in nifedipine molecule has been fixed. We think that this orientation of molecule can be important to bind to the channel. Active calcium channel antagonists carrying 1,4-DHP structure 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. The introduction of second atom into aromatic ring increases biological activity and decreases toxicity is well known [10]. In this study, we aimed to search the calcium antagonistic activity of 7-substituted HHQ derivatives with dichloro substituents.

KEY WORDS: Calcium antagonistic activity, Hexahydroquinoline, Nicardipine, Synthesis.

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 ISSN 0326-2383

MATERIAL AND METHODS

Chemistry

All compounds objected in this study were sythesized before following procedure:

Synthesis of methyl(ethyl) 4-(dichlorophenyl)-2,7-dimethyl-5-oxo-l,4,5,6,7,8-hexahydroqui-noline-3-carboxylates (3a-p)

A mixture of methyl or ethyl aminocrotonate (0,001 mol) 5-methyl (phenyl)-1,3-cyclohexanedione (0,001 mol) and appropriate aromatic aldehyde (0,001 mol) in 20 mL methanol was refluxed for 4 h. The solvent was evaporated and the residue was crystallized from alcohol.

Pharmacology

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

Studies on isolated rat ileum [11]

Albino rats of either sex weighing (150-200 g) were used in the present study. In pharmacological studies, one animal was used for each compound. They were supplied from Laboratory Animal Production Center in Department of Pharmacology, School of Medicine, Osmangazi University, Eski?ehir (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, discarding the 5-8 cm segment proximal to the ileocaecal junction. 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% O2 and 5% CO_2. A tension of 2 g was applied and isometric recording was done by using 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. regular washes every 15 min. In order to check for calcium antagonistic effects, contractions were induced with barium chloride (3.10^{-3} mol/1, bath concentration). After washing out, this process was repeated until the amplitude of the contraction become 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 (DMSO) at different concentrations (10^{-8} - 10^{-5} M) and 5 min exposure time. Only one compound was tested in each preparation. Control experiments were performed by using DMSO 5 min exposure time. The results were evaluated after subtraction of the control from the test results to eliminate the effects of DMSO. EC50 values were calculated from individual dose-response curves automatically by data acquisiton system.

Studies on rat thoracic aorta [11, 12]

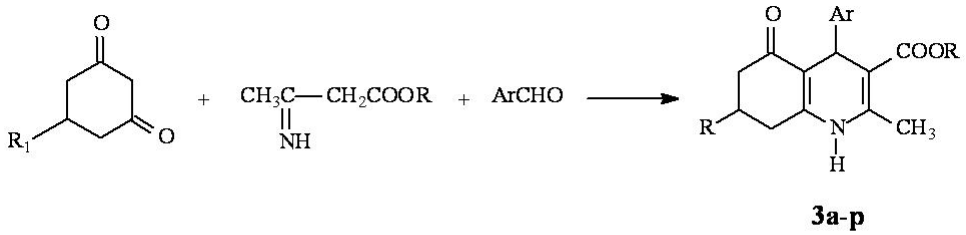
Rings (3 mm) were prepared using rat thoracic aorta and they were suspended in organ baths of 10 mL capacity which contained Tyrode solution. The bath contents were maintained at 37 °C and aerated by 95% O_2 and 5% CO_2. in a gas of ± 95% O_2 and 5% CO_2 and a tension of 2 g was applied. The preparations 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 become constant. Investigations of the substances were performed using the single dose technique. Potassium chloride contractions were induced after addition of the test substance (10^{-7}- 10^{-4} M) and 10 min exposure time. During the 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).

RESULTS AND DISCUSSION

The HHQ derivatives were prepared by the Hantzsch reaction [13]. The reaction of a dichlorobenzaldehyde derivative, methyl(ethyl) aminocrotonate and 5-methyl (or 5-phenyl)-l,3-cyclohexanedione in methanol yielded the respective HHQ derivative.

The formula of the compounds are given in Table 1. The compounds were published with their relaxant effects on isolated rabbit gastric fundus [14].

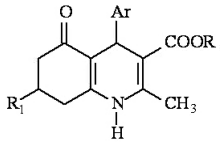
In addition, X-ray analysis of two compounds (compounds **3c** and **3k**) was realised and published in elsewhere [15]. X-ray analysis results showed that the 2,3-dichlorophenyl ring is



3a-p

R_1: CH_3, C_6H_5
R: CH_3, C_2H_5
Ar: Dichlorophenyl

Figure 2. Synthesis of HHQ derivatives.



Compound	R	R1	Ar	Mp (°C)	Analysis (CHN) calc/found
3a	CH_3	CH_3	2,3-dichlorophenyl	245	60.01, 5.04, 3.68 59.91, 5.32, 3.78
3b	C_2H_5	CH_3	2,3-dichlorophenyl	246	60.92, 5.37, 3.55 60.36, 5.30, 3.63
3c	CH_3	CH_3	2,4-dichlorophenyl	250	60.01, 5.04, 3.68 59.53, 4.90, 3.78
3d	C_2H_5	CH_3	2,4-dichlorophenyl	218	60.92, 5.37, 3.55 60.74, 5.33, 3.63
3e	CH_3	CH_3	2,5-dichlorophenyl	229	60.01, 5.04, 3.68 59.92, 5.07, 3.73
3f	C_2H_5	CH_3	2,5-dichlorophenyl	236	60.92, 5.37, 3,55 60.26, 5.20, 3.67
3g	CH_3	CH_3	2,6-dichlorophenyl	243	60.01, 5.04, 3.68 59.41, 4.99, 3.68
3h	C_2H_5	CH_3	2,6-dichlorophenyl	244	60.92, 5.37, 3.55 60.31, 5.34, 3.65
3i	CH_3	C_6H_5	2,3-dichlorophenyl	228	65.17, 4.79, 3.17 64.49, 4.40, 3.16
3j	C_2H_5	C_6H_5	2,3-dichlorophenyl	131	65.80, 5.08, 3.07 65.94, 4.87, 2.73
3k	CH_3	C_6H_5	2,4-dichlorophenyl	148	65.17, 4.79, 3.17 65.46, 5.07, 3.09
3l	C_2H_5	C_6H_5	2,4-dichlorophenyl	130	65.80, 5.08, 3,07 65.91, 5.47, 2.93
3m	CH_3	C_6H_5	2,5-dichlorophenyl	184	65.17, 4.79, 3,17 65.88, 4.69, 3.16
3n	C_2H_5	C_6H_5	2,5-dichlorophenyl	129	65.80, 5.08, 3,07 65.30, 4.89, 2.82
3o	CH_3	C_6H_5	2,6-dichlorophenyl	128	65.17, 4.79, 3.17 65.31, 4.60, 3.43
3p	C_2H_5	C_6H_5	2,6-dichlorophenyl	113	65.80, 5.08, 3.07 65.10, 5.03, 2.61

Table 1. Synthesized compounds.

	% Inhibition				
	10^{-8} M	**10^{-7} M**	**10^{-6} M**	**5.10^{-6} M**	**10^{-5} M**
3a	73.60 ± 26.33	-	-	-	-
3b	-	15.42 ± 5.34	57.83 ± 13.38	74.17 ± 6.74	91.33 ± 2.94
3c	8.58 ± 6.97*	13.00 ± 5.85	24.00 ± 13.5	-	31.66 ± 18.66
3d	15.40 ± 2.30*	21.80 ± 3.70	40.00 ± 6.52	44.00 ± 6.89	56.80 ± 7.46
3e	55.75 ± 8.40*	75.00 ± 8.71	84.17 ± 4.26	-	-
3f	39.33 ± 16.51*	66.33 ± 11.66	73.50 ± 8.38	73.50 ± 9.62	-
3g	-	47.50 ± 10.74	83.80 ± 8.64	-	-
3h	12.40 ± 5.22*	18.00 ± 8.06	28.00 ± 14.54	-	29.50 ±9.44
3i	11.50 ± 5.68*	31.50 ± 9.08	60.00 ± 14.79	77.83 ± 7.68	81.25 15.07
3j	0	19.75 ± 10.30	32.60 ± 17.08	-	34.83 ±10.93
3k	0	10.33 ± 3.02	21.25 ± 6.27	-	43.75 ± 17.97
3l	0	0	13.53 ± 7.57	-	18.40 ± 6.03
3m	0	12.25 ± 8.54	47.25 ± 10.82	65.75 ± 8.58	90.50 ± 5.21
3n	-	19.38 ± 8.18	52.13 ± 5.64	84.25 ± 7.22	-
3o	-	16.88 ± 3.98	46.00 ± 7.54	69.82 ± 17.10	80.50 ± 13.78
3p	-	28.50 ± 12.18	53.50 ± 13.37	74.13 ± 8.25	84.83 ± 8.04
Nicardipine	82.50±3.42	100			

Table 2. Relaxant effects of the compounds and nicardipine on isolated rat ileum precontracted with barium chloride (4x 10^{-3} mol/1) (% inhibition ± SD), (n=6). * $p<0.05$ significantly different from nicardipine group.

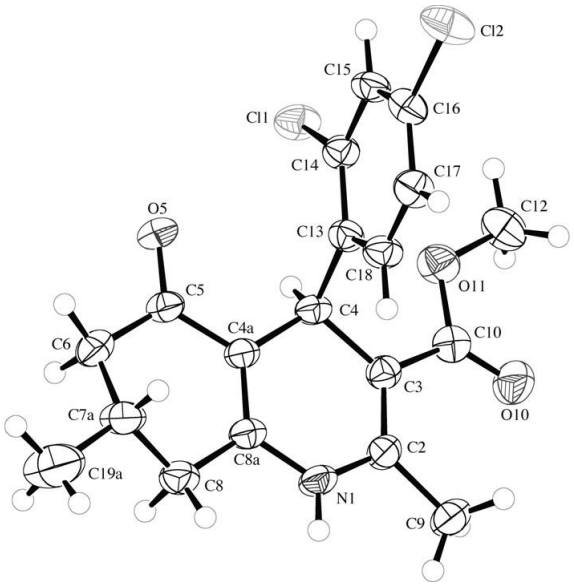


Figure 3. X-Ray diagram of compound **3c**.

Compound	EC50
3a	$6.40 \pm 2.30.10^{-9}$ *
3b	$1.83 \pm 0.76.10^{-8}$ *
3d	$5.18 \pm 2.84.10^{-6}$ *
3e	$1.25 \pm 0.61.10^{-8}$ *
3f	$5.35 \pm 4.06.10^{-8}$ *
3g	$2.92 \pm 1.20.10^{-7}$ *
3i	$7.58 \pm 4.93.10^{-7}$ *
3m	$8.80 \pm 2.50.10^{-7}$ *
3n	$2.25 \pm 1.25.10^{-6}$ *
3o	$2.25 \pm 1.89.10^{-6}$ *
3p	$1.28 \pm 0.62.10^{-6}$ *
Nicardipine	$7.20 \pm 2.58.10^{-10}$

Table 3. EC_{50} values of compounds and nicardipine in isolated rat ileum. (n = 5)*; $p < 0.05$ significantly different from nicardipine group.

oriented such that the chloro substituents are in a synperiplanar orientation with respect to the 1,4-DHP ring plane and the oxocyclohexene ring has a slightly distorted envelope conformation (Fig. 3). Both structures exhibit the same intermolecular N---H....O hydrogen bonding motif in which the molecules are linked into chains by interactions involving the carbonyl oxygen atom of the oxocyclohexene ring. Finally, the elemental analysis results are also consistent with the postulated structures.

Calcium antagonistic activities of the compounds were determined by the tests performed on isolated rat ileum and rat thorasic aorta. In activity test nicardipine was used as the standard. In the studies on isolated rat ileum, it has

Compound	% Inhibition		
	10^{-6} M	10^{-5} M	10^{-4} M
3a	18.08 ± 4.31	26.91 ± 8.81	32.45 ± 11.80
3b	4.53 ± 1.79*	8.20 ± 2.94*	11.63 ± 2.78*
3e	5.52 ± 2.18*	15.38 ± 7.25	23.45 ± 5.27
3f	14.48 ± 7.34	23.50 ± 11.21	29.50 ± 14.93
3g	5.59 ± 2.21*	13.18 ± 4.99	-
3i	0	4.02 ± 2.84*	20.79 ± 9.62
3m	0	4.91 ± 2.75*	18.32 ± 3.53*
3n	8.59 ± 3.74*	11.12 ± 5.55	27.56 ± 13.04
3o	0	3.60 ± 2.21*	19.45 ± 8.81*
3p	2.42 ± 1.59*	6.04 ± 3.44*	8.79 ± 2.70*
Nicardipine	25.60 ± 14.06	25.89 ± 15.56	36.69 ± 15.95

Table 4. Relaxant effects of the compounds and nicardipine on thoracic aorta precontracted with potassium chloride (67 mmol/L) (% inhibition ± SD) (n= 6). *:$p<0.05$ significantly different from nicardipine group.

been observed that all compounds showed meaningfull activity in some concentrations except compounds **3c**, **3h**, **3j-l** (Table 2). When the obtained results were analysed in respect to EC_{50} values of the compounds, it has been seen that 7-methyl derivatives

(**3b**, **3e**, **3f**) have generally lower EC50 values than 7-phenyl derivatives (Table 3). In addition, the compounds having 2,3-dichlorophenyl ring have been found as the most active ones. In the studies on rat thoracic aorta, Compound **3a** has been found as active as nicardipine in 10^{-8} M concentration. There was no significant difference between the effects of nicardipine and that of **3a** (Table 4).

CONCLUSION

The compounds having hexahydroquinoline structure were synthesized and their calcium channel modulatory activity elucidated on isolated tissue. All compounds showed mentioned activity. Compound **3a** is more active than nicardipine on rat thorasic aorta. Especially 7-methyl derivatives

(**3b**, **3e**, **3f**) have generally lower EC_{50} values than 7-phenyl derivatives. In addition, 2,3-dichlorophenyl analogs are the most active ones. Also, compound **3a** has been found as active as nicardipine in 10^{-8} M concentration on rat thoracic aorta.

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