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Original article

Evaluation of myorelaxant activity of 7-substituted hexahydroquinoline derivatives in isolated rabbit gastric fundus

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Abstract

In this article, 16 new methyl(ethyl) 4-(dichlorophenyl)-2,7-dimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylates and methyl(ethyl) 2-methyl-4-(dichlorophenyl)-5-oxo-7-phenyl-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives have been synthesized by the Hantzsch reaction and screened for their myorelaxant and potassium channel opening activities. The maximum relaxant effects (E_{max}) and pD_2 values on exogenous noradrenaline precontracted tissues and inhibitory effects on cholinergic neurotransmission of the compounds and pinacidil were determined on isolated strips of rabbit gastric fundus smooth muscle. Obtained results indicated that some compounds and pinacidil produced concentration-dependent relaxation on rabbit gastric fundus smooth muscle strips in the two test conditions.

Keywords: Hexahydroquinoline; Myorelaxant activity; Potassium channel opener; Cholinergic neurotransmission; Rabbit gastric fundus

1. Introduction

It is well known that ion channels are very important for cell function and responsible for physiological effects. Potassium channels regulate some functions in both excitable and non-excitable cells. Potassium channel opening is a physiological mechanism by which excitable cells exploit to maintain or restore their resting state [1–5].

K_{ATP} channel (ATP-sensitive K^+ channel) openers are a structurally diverse group of drugs with a broad spectrum of potential therapeutic usages. These drugs interact with K_{ATP} channels in numerous tissues and increase their activity, thereby hyperpolarizing the plasma membrane and reducing electrical excitability [6]. These channels play critical roles to modulate physiological processes such as insulin secretion, leptin release, synaptic transmission and excitability of cardiac, vascular and nonvascular smooth muscles [7].

K_{ATP} channel openers have a relaxation effect due to lower cellular membrane potential and inhibit calcium influx. There has been considerable interest in exploring K_{ATP} channel openers in the treatment of various diseases such as cardiovascular, cerebrovascular, and urinary system disease and premature labor [8,9].

The properties of K_{ATP} channels in guinea pig gastric myocytes were similar to those of K_{ATP} channels in other smooth muscles [10].

Many 1,4-dihydropyridine (1,4-DHP) derivatives are known to be calcium channel blockers. In addition it has been shown that some of these derivatives have potassium channel opening activity [11]. For example, niguldipine, which is a 1,4-DHP derivative has increased K^+ flux in isolated vascular smooth muscle by opening Ca^{2+}-activated potassium channels [3].

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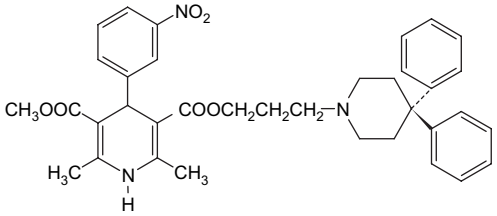


Niguldipine

It is well known that bicyclo (quinoline) and tricyclo (acridine) analogs of 1,4-DHP also have potassium channel opening activities [12,13]. The object of this study is to investigate the effects of quinoline derivatives in rabbit gastric fundus smooth muscle strips.

In this article, 16 annelated 1,4-DHP derivatives have been synthesized to take into one of the carbonyl groups in condensed ring system. The contribution of dichlorophenyl in 4 position and methyl or phenyl substituent in 7 position of quinoline ring to the activity was also studied.

2. Results and discussion

2.1. Chemistry

The hexahydroquinoline (HHQ) derivatives were prepared by the Hantzsch reaction [14]. The reaction of a dichlorobenzaldehyde derivative, alkyl aminocrotonate and 5-methyl (or 5-phenyl)-l,3-cyclohexanedione in methanol yielded the respective HHQ derivative (Scheme 1).

The structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis. In the IR spectra, characteristic N–H and C=O stretching bands were seen. In ^{1}H NMR spectra, 7-CH_3 protons of the HHQ ring were seen at 1.05 ppm for compounds **3a–i**. H-7 protons of all compounds, were seen at 2.00–3.00 ppm. 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 8.80 ppm as a broad singlet. The ^{13}C NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of carbon atoms. The mass spectra of the compounds were recorded using the electron impact technique. Molecular ion peaks were seen for all molecules. Also the base peak is formed 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 analog was also observed (Scheme 2). These findings are in accordance with the literature [15–17].

In addition, X-ray analysis of compounds **3c** and **3k** was realised and published elsewhere [18]. X-ray analysis results showed that the 2,3-dichlorophenyl ring is 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. 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.

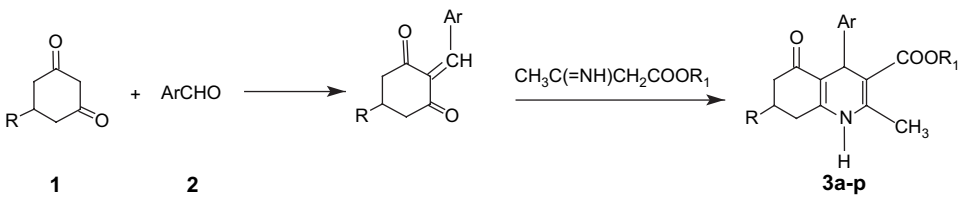
Although all compounds have two asymmetric centers in their 4 and 7 positions of HHQ ring, their isomers could not be separated due to technical shortage. Therefore the effect of isomerism on the mentioned activity could not be determined.

2.2. Pharmacology

2.2.1. Relaxant effects of compounds and pinacidil on the tissues precontracted by noradrenaline

The maximum relaxant effects (E_{max}) and pD_2 values of compounds **3a–p** and pinacidil on isolated strips of rabbit gastric fundus smooth muscle are given in Table 1.

The results indicated that compounds **3a–j**, **3l**, **3m** produced concentration-dependent relaxation in rabbit gastric fundus smooth muscle strips. Compounds **3k**, **3n–p** displayed slightly relaxant responses which were not statistically significant from control relaxations produced by dimethylsulphoxide (DMSO). Compounds and pinacidil exerted concentration-dependent relaxation responses precontracted with submaximal concentration of noradrenaline in the gastric fundus smooth muscle strips with the efficacy order: **3b** > **3l** ≥ **3c** ≥ pinacidil ≥ **3m** ≥ **3j** > **3d** > **3h** > **3i** ≥ **3g** > **3a** > **3e** ≥ **3f**. It is interesting that E_{max} value of compound **3b** was higher than that of pinacidil. Also E_{max} values of compounds **3c** and **3l** have been found similar to that of pinacidil. Glibenclamide did not reverse the myorelaxant effects of the



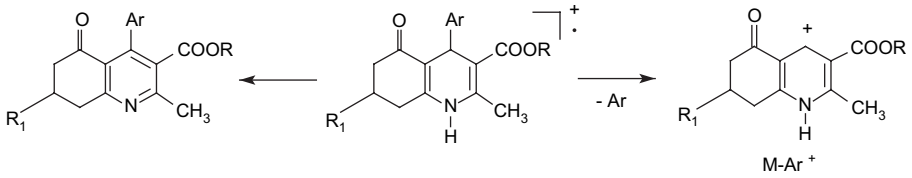
1 **2** **3a-p**

R: CH_3, C_6H_5

R_1: CH_3, C_2H_5

Ar: Dichlorophenyl

Scheme 1. Synthesis of HHQ derivatives.



Scheme 2. Main fragmentation of the compounds.

compounds. These results suggested that myorelaxant effects of the compounds were not mediated by K_{ATP} channels. Calcium channels or other types of potassium channels may mediate these effects of the compounds. While compounds **3a**, **3i** and **3l** had a direct relaxant effect on exogenous noradrenaline precontracted tissues, they did not alter EFS induced contractile responses (Table 1).

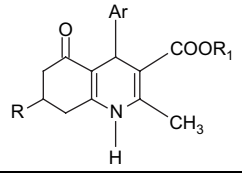
To investigate whether relaxation induced by the test compounds was due to interaction with the cyclooxygenase, adrenergic system or nitric oxide pathways, tissues were pretreated with indomethacin (cyclooxygenase inhibitor), propranolol (β-adrenergic receptor blocker) or *N*-ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor), respectively. Pretreatment of the strips with indomethacin, propranolol and L-NAME did not significantly alter the relaxant responses to the compounds. This situation explained that cyclooxygenase, adrenergic and nitric oxide (NO) pathways do not play a role in relaxations evoked by these substances.

2.2.2. Effects of compounds and pinacidil on EFS evoked neurogenic contractions of the rabbit gastric fundus smooth muscle strips

Pharmacological analysis of field stimulation-induced mechanical responses in isolated smooth muscle strips is a useful in vitro technique for clarifying neurotransmitter release. Presynaptic potassium channels play an important role on the EFS evoked neurotransmitter release. EFS evoked contractile responses in rabbit gastric fundus. Tetrodotoxin (TTX) abolished these responses. Cadmium (Cd^{2+}) ions reduced the contraction responses evoked by EFS. In this study, atropine (a muscarinic receptor blocker), abolished the contraction responses evoked by EFS and neostigmine (acetylcholinesterase inhibitor), potentiated responses indicating that EFS evoked contractile responses due to cholinergic neurotransmission.

Compounds **3b–3h**, **3j**, **3k**, **3m**, **3o**, **3p** and pinacidil caused a concentration-dependent inhibition of EFS evoked contractions of rabbit gastric fundus smooth muscle strips as given in Table 1.

Table 1
Maximum relaxant responses (E_{max}*) and pD_2* values on precontracted tissues by noradrenaline and maximum inhibitory responses (E_{max}**) and pD_2** values of compounds **3a–p** and pinacidil on EFS evoked contractile responses on isolated strips of rabbit gastric fundus smooth muscle



	R	R_1	Ar	E_{max}*	pD_2*	E_{max}**	pD_2**
3a	CH_3	CH_3	2,3-Dichlorophenyl	53.99 ± 13.01[a]	5.26 ± 0.58[a]	No effect	No effect
3b	CH_3	C_2H_5	2,3-Dichlorophenyl	89.47 ± 8.77[a]	6.02 ± 0.68[a]	52.87 ± 12.08[a]	6.02 ± 0.62[a]
3c	CH_3	CH_3	2,4-Dichlorophenyl	71.2 ± 9.41[a]	5.62 ± 0.76[a]	57.71 ± 3.30[a]	5.23 ± 0.79[a]
3d	CH_3	C_2H_5	2,4-Dichlorophenyl	63.65 ± 5.41[a]	5.96 ± 0.27[a]	52.04 ± 8.62[a]	5.27 ± 0.32[a]
3e	CH_3	CH_3	2,5-Dichlorophenyl	47.99 ± 3.81[a]	5.54 ± 0.31[a]	45.95 ± 21.69[a]	5.03 ± 0.36[a]
3f	CH_3	C_2H_5	2,5-Dichlorophenyl	45.78 ± 5.99[a]	5.75 ± 0.36[a]	47.74 ± 4.40[a]	6.16 ± 0.38[a]
3g	CH_3	CH_3	2,6-Dichlorophenyl	56.01 ± 9.55[a]	5.91 ± 0.61[a]	69.62 ± 1.29[a]	6.26 ± 0.55[a]
3h	CH_3	C_2H_5	2,6-Dichlorophenyl	59.75 ± 6.12[a]	5.93 ± 0.23[a]	52.07 ± 21.31[a]	6.05 ± 0.27[a]
3i	C_6H_5	CH_3	2,3-Dichlorophenyl	56.29 ± 5.89[a]	5.62 ± 0.46[a]	No effect	No effect
3j	C_6H_5	C_2H_5	2,3-Dichlorophenyl	66.35 ± 13.95[a]	6.06 ± 0.73[a]	41.25 ± 12.17[a]	5.15 ± 0.69[a]
3k	C_6H_5	CH_3	2,4-Dichlorophenyl	No effect	No effect	41.31 ± 4.84[a]	6.35 ± 0.47[a]
3l	C_6H_5	C_2H_5	2,4-Dichlorophenyl	72.46 ± 12.61[a]	7.06 ± 0.93[a]	No effect	No effect
3m	C_6H_5	CH_3	2,5-Dichlorophenyl	67.72 ± 13.76[a]	6.54 ± 1.06[a]	53.56 ± 11.29[a]	7.05 ± 0.97[a]
3n	C_6H_5	C_2H_5	2,5-Dichlorophenyl	No effect	No effect	No effect	No effect
3o	C_6H_5	CH_3	2,6-Dichlorophenyl	No effect	No effect	88.61 ± 5.87[a]	5.35 ± 0.38[a]
3p	C_6H_5	C_2H_5	2,6-Dichlorophenyl	No effect	No effect	75.08 ± 8.45[a]	5.05 ± 0.57[a]
Pinacidil				71.15 ± 5.39[a]	5.03 ± 0.12[a]	76.33 ± 3.44[a]	7.49 ± 0.15[a]

Relaxation is expressed as a percentage of the precontraction induced by noradrenaline. The inhibitory effects of the compounds and pinacidil on EFS evoked contractions were expressed as percentage of the control contraction. The negative logarithm of the concentration for the half-maximal response (pD_2) and E_{max} values represent mean ± S.E.

[a] $p < 0.05$, compared with control responses ($n = 6$).

Compounds **3a**, **3i**, **3l** and **3n** displayed inhibitory responses, which were not statistically significant from control inhibition produced by DMSO. Compounds and pinacidil exerted concentration-dependent inhibition of EFS induced contractile responses in the gastric fundus smooth muscle strips with the efficacy order: **3o** > pinacidil = **3p** > **3g** > **3c** ≥ **3m** = **3b** = **3d** = **3h** ≥ **3f** ≥ **3e** ≥ **3k** = **3j**.

It is interesting to note that efficacy of compound **3o** is greater than that of pinacidil but potency of this compound is lower than that of pinacidil. Efficacy of compound **3p** is similar to that of pinacidil but pinacidil is 100 fold more potent than this compound (Table 1). In our study, Glibenclamide, a K_{ATP} channel blocker, at 10^{-6} M reversed the inhibition effects of compounds **3c** (16.14%), **3e** (11.24%), **3g** (27.35%), **3h** (17.16%) and pinacidil (32.18%) on EFS evoked contractions. This result suggests that inhibitory effects of mentioned compounds were possibly mediated, at least in part, by presynaptic K_{ATP} channels. Surprisingly, compounds **3o** and **3p** which had no direct relaxant effect on exogenous noradrenaline precontractile tissues, have inhibited EFS induced contractile responses. On the other hand, it is also surprising to note that the most potent compound on isolated strips of rabbit gastric fundus smooth muscle (compound **3l** with a pD_2 value of 7.06) was completely inactive on EFS evoked neurogenic contraction of the same smooth muscle.

3. Conclusion

Our results showed that several hexahydroquinoline derivatives have a myorelaxant activity in isolated rabbit gastric fundus smooth muscle precontracted by noradrenaline similar to pinacidil. Glibenclamide did not reverse the myorelaxant effects of the compounds. This result suggests that myorelaxant effects of compounds were not mediated by K_{ATP} channels. Several other compounds have an inhibitory activity on the EFS evoked contractions (cholinergic neurotransmission) in isolated rabbit gastric fundus smooth muscle similar to pinacidil. Glibenclamide partially reversed the inhibitory effects of compounds **3c**, **3e**, **3g**, **3h** and pinacidil on EFS evoked contractions. This result suggests that inhibitory effects of compounds **3c**, **3e**, **3g** and **3h** were possibly mediated at least in part by K_{ATP} channels. But the potassium channel opening ability of these compounds needs further investigations by using radioisotopic or electrophysiological experiments.

4. Experimental

4.1. Chemistry

All chemicals used in this study were purchased from Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland) and Sigma (Germany).

Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and are uncorrected. IR spectra: Perkin Elmer FT-IR Spectrophotometer 1720 X (Beaconsfield, UK) (KBr disc) (ν, cm^{-1}). ^1H NMR Spectra: Bruker GMB HD PX-400 MHz Digital FT NMR and ^1H AMX 600 MHz FT NMR Spectrophotometer (Karlsruhe, Germany) (DMSO-d_6; tetramethylsilane as internal standard). ^{13}C NMR Spectra: ^{13}C AMX 150 MHz FT NMR Spectrophotometer. Chemical shift values are given as ppm. Mass spectra: Hewlett Packard Series II Plus 5890 GAS Chromatograph–Hewlett Packard 5972 Series Mass Selective Detector (Philadelphia, USA). Thin Layer Chromatography (TLC) was run on precoated silica gel (E. Merck, Darmstadt, Germany) and short wave UV light (254 nm) was used to detect the UV absorbing spots. Elemental analysis was carried out on a Leco 932 CHNS-O Elemental Analyzer (Philadelphia, USA) (TUBITAK, Ankara, Turkey). The elemental analysis results were within 0.4% of theoretical values.

4.1.1. Synthesis of methyl(ethyl) 4-(dichlorophenyl)-2,7-dimethyl-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylates (**3a–h**)

A mixture of methyl or ethyl aminocrotonate (0.001 mol) 5-methyl-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.

*4.1.1.1. Methyl 4-(2,3-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3a**).* Mp. 245 °C. IR (cm^{-1}) 3288, 1703, 1616, 776. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 2.05–2.70 (m, 5H, *H*-6, 7, 8 HHQ), 2.35 (s, 3H, 2-CH_3), 3.60 (s, 3H, COOCH_3), 5.41 (s, 1H, *H*-4 HHQ), 7.00–7.50 (m, 3H, *Ar*), 8.90 (broad, 1H, N*H*). ^{13}C NMR δ 19.1, 20.7, 28.8, 35.2, 36.9, 51.0, 53.3, 102.3, 106.1, 126.7, 127.3, 128.4, 131.7, 133.1, 133.7, 143.5, 146.0, 167.3, 197.4. MS (*m/z*) 234 (%100). Anal. calcd. ($C_{19}H_{19}Cl_2NO_3$) C, H, N.

*4.1.1.2. Ethyl 4-(2,3-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3b**).* Mp. 246 °C. IR (cm^{-1}) 3290, 1702, 1616, 769. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 1.20 (t, 3H, CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 2.00–2.55 (m, 5H, *H*-6,7,8 HHQ), 4.05 (q, 2H, CH_2CH_3), 5.45 (s, 1H, *H*-4 HHQ), 7.00–7.40 (m, 3H, *Ar*), 8.90 (broad, 1H, N*H*). ^{13}C NMR δ 14.2, 19.2, 20.7, 28.8, 35.3, 35.5, 37.2, 59.9, 103.1, 106.0, 126.5, 126.6, 128.3, 130.5, 131.8, 133.0, 143.7, 145.2, 167.0, 195.7. MS (*m/z*) 248 (%100). Anal. calcd. ($C_{20}H_{21}Cl_2NO_3$) C, H, N.

*4.1.1.3. Methyl 4-(2,4-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3c**).* Mp. 250 °C. IR (cm^{-1}) 3284, 1708, 1615, 761. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 2.00–2.55 (m, 5H, *H*-6,7,8 HHQ), 2.35 (s, 3H, 2-CH_3), 3.60 (s, 3H, COOCH_3), 5.35 (s, 1H, *H*-4 HHQ), 7.05–7.35 (m, 3H, *Ar*), 8.90 (broad, 1H, N*H*). ^{13}C NMR δ 19.0, 20.8, 28.7, 35.2, 44.7, 50.9, 53.4, 104.9, 105.1, 126.8, 127.1, 129.1, 132.1, 132.6, 133.7, 143.2, 144.3, 167.7, 196.1. MS: (*m/z*) 234 (%100). Anal. calcd. ($C_{19}H_{19}Cl_2NO_3$) C, H, N.

*4.1.1.4. Ethyl 4-(2,4-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3d**).* Mp. 218 °C. IR

(cm^{-1}) 3275, 1703, 1608, 761. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 1.20 (t, 3H, t; CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 2.00–2.55 (m, 5H, *H*-6,7,8 HHQ), 4.10 (q, 2H, CH_2CH_3), 5.35 (s, 1H, *H*-4 HHQ), 7.00–7.40 (m, 3H, *Ar*), 8.85 (broad, 1H, N*H*). ^{13}C NMR δ 14.2, 19.3, 20.6, 28.8, 35.3, 35.9, 44.7, 60.0, 111.2, 111.4, 126.7, 129.3, 132.2, 133.1, 133.3, 133.9, 142.6, 143.7, 167.1, 195.7. MS: (*m/z*) 248 (%100). Anal. calcd. ($C_{20}H_{21}Cl_2NO_3$) C, H, N.

*4.1.1.5. Methyl 4-(2,5-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3e**).* Mp. 229 °C. IR (cm^{-1}) 3282, 1701, 1611, 741. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 2.00–2.60 (m, 5H, *H*-6,7,8 HHQ), 2.40 (s, 3H, 2-CH_3), 3.60 (s, 3H, COOCH_3), 5.35 (s, 1H, *H*-4 HHQ), 7.00–7.35 (m, 3H, *Ar*), 8.95 (broad, 1H, N*H*). ^{13}C NMR δ 19.2, 20.7, 28.9, 35.3, 35.8, 44.5, 50.9, 105.8, 111.7, 127.5, 130.4, 130.6, 131.2, 131.7, 132.3, 143.7, 145.9, 167.4, 195.6. MS: (*m/z*) 234 (%100). Anal. calcd. ($C_{19}H_{19}Cl_2NO_3$) C, H, N.

*4.1.1.6. Ethyl 4-(2,5-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3f**).* Mp. 236 °C. IR (cm^{-1}) 3285, 1699, 1612, 746. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 1.15 (t, 3H, CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 2.00–2.60 (m, 5H, *H*-6,7,8 HHQ), 4.05 (q, 2H, CH_2CH_3), 5.35 (s, 1H, *H*-4 HHQ), 7.00–7.35 (m, 3H, *Ar*), 8.95 (broad, 1H, N*H*). ^{13}C NMR δ 14.1, 19.3, 20.8, 28.9, 35.3, 36.3, 44.6, 60.0, 111.1, 111.3, 127.4, 129.2, 130.6, 130.9, 131.8, 132.1, 143.8, 145.7, 167.0, 195.7. MS (*m/z*) 248 (%100). Anal. calcd. ($C_{20}H_{21}Cl_2NO_3$) C, H, N.

*4.1.1.7. Methyl 4-(2,6-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3g**).* Mp. 243 °C. IR (cm^{-1}) 3301, 1704, 1616, 780. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 1.95–2.50 (m, 5H, *H*-6,7,8 HHQ), 2.25 (s, 3H, 2-CH_3), 3.55 (s, 3H, COOCH_3), 5.85 (s, 1H, *H*-4 HHQ), 6.90–7.35 (m, 3H, *Ar*), 9.05 (broad, 1H, N*H*). ^{13}C NMR δ 18.9, 20.7, 28.5, 35.1, 35.6, 43.9, 50.6, 103.1, 106.8, 127.3, 132.1, 133.3, 138.8, 144.8, 145.3, 167.6, 195.3. MS (*m/z*) 234 (%100). Anal. calcd. ($C_{19}H_{19}Cl_2NO_3$) C, H, N.

*4.1.1.8. Ethyl 4-(2,6-dichlorophenyl)-2,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3h**).* Mp. 244 °C. IR (cm^{-1}) 3292, 1703, 1652, 776. ^1H NMR δ 1.05 (d, 3H, 7-CH_3), 1.15 (t, 3H, CH_2CH_3), 2.25 (s, 3H, 2-CH_3), 1.95–2.65 (m, 5H, *H*-6,7,8 HHQ), 4.05 (q, 2H, CH_2CH_3), 5.85 (s, 1H, *H*-4 HHQ), 6.95–7.45 (m, 3H, *Ar*), 9.05 (broad, 1H, N*H*). ^{13}C NMR δ 14.1, 19.1, 20.7, 28.5, 35.2, 35.5, 49.6, 59.8, 103.6, 106.8, 127.4, 132.1, 132.3, 138.6, 143.9, 145.7, 166.9, 194.8. MS (*m/z*) 248 (%100). Anal. Calcd. ($C_{20}H_{21}Cl_2NO_3$) C, H, N.

*4.1.2. Synthesis of methyl(ethyl) 4-(dichlorophenyl)-2-methyl-5-oxo-7-phenyl-l,4,5,6,7,8-hexahydroquinoline-3-carboxylates (**3i–p**)*

A mixture of methyl(ethyl) aminocrotonate (0.001 mol) 5-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.

*4.1.2.1. Methyl 4-(2,3-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3i**).* Mp. 228 °C. IR (cm^{-1}) 3292, 1707, 1612, 740. ^1H NMR δ 2.35 (s, 3H, 2-CH_3), 2.45–2.85 (m, 5H, *H*-6,7,8 HHQ), 3.60 (s, 3H, COOCH_3), 5.45 (s, 1H, *H*-4 HHQ), 7.00–7.45 (m, 8H, *Ar*), 8.65 (broad, 1H, N*H*). ^{13}C NMR δ 19.3, 34.9, 37.1, 39.3, 43.6, 50.9, 104.9, 112.2, 126.7, 127.1, 127.3, 128.9, 130.0, 130.2, 131.8, 133.1, 142.4, 146.2, 149.4, 151.2, 167.6,194.7. MS (*m/z*) 296 (%100). Anal. calcd. ($C_{24}H_{21}Cl_2NO_3$) C, H, N.

*4.1.2.2. Ethyl 4-(2,3-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3j**).* Mp.131 °C. IR (cm^{-1}) 3292, 1699, 1614, 765. ^1H NMR δ 1.15 (t, 3H, CH_2CH_3), 2.30 (s, 3H, 2-CH_3), 2.45–2.85 (m, 5H, *H*-6,7,8 HHQ), 4.05 (q, 2H, CH_2CH_3), 5.45 (s, 1H, *H*-4 HHQ), 7.00–7.45 (m, 8H, *Ar*), 8.65 (broad, 1H, N*H*). ^{13}C NMR δ 14.2, 19.2, 34.6, 37.2, 39.3, 43.6, 59.9, 105.6, 111.4, 126.5, 126.7, 127.1, 128.7, 130.4, 131.1 131.8, 133.0, 142.3, 143.6, 145.9, 146.1, 167.1, 194.9. MS (*m/z*) 310 (%100). Anal. Calcd. ($C_{25}H_{23}Cl_2NO_3$) C, H, N.

*4.1.2.3. Methyl 4-(2,4-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3k**).* Mp. 148 °C. IR (cm^{-1}) 3303, 1704, 1618, 766. ^1H NMR δ 2.30 (s, 3H, 2-CH_3), 2.45–2.85 (m, 5H, *H*-6,7,8 HHQ), 3.60 (s, 3H, COOCH_3), 5.40 (s, 1H, *H*-4 HHQ), 7.00–7.45 (m, 8H, *Ar*), 8.65 (broad, 1H, N*H*). ^{13}C NMR δ 19.0, 34.5, 38.8, 39.3, 43.5, 50.9, 105.3, 111.6, 126.7, 127.1, 127.4, 128.9, 129.1, 132.1, 132.6, 133.1, 133.8, 142.3, 143.1, 144.3, 167.6, 195.1. MS (*m/z*) 296 (%100). Anal. calcd. ($C_{24}H_{21}Cl_2NO_3$) C, H, N.

*4.1.2.4. Ethyl 4-(2,4-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3l**).* Mp. 130 °C. IR (cm^{-1}) 3293, 1702, 1618, 766. ^1H NMR δ 1.25 (t, 3H, CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 2.45–2.85 (m, 5H, *H*-6,7,8 HHQ), 4.10 (q, 2H, CH_2CH_3), 5.40 (s, 1H, *H*-4 HHQ), 7.00–7.55 (m, 8H, *Ar*), 8.65 (broad, 1H, N*H*). ^{13}C NMR δ 14.2, 19.3, 34.9, 38.7, 39.4, 43.6, 59.9, 104.9, 111.7, 126.7, 126.9, 127.1, 128.7, 129.2, 132.9, 133.1, 134.0, 142.3, 143.6, 149.7, 158.9, 167.1, 194.7. MS (*m/z*) 310 (%100). Anal. calcd. ($C_{25}H_{23}Cl_2NO_3$) C, H, N.

*4.1.2.5. Methyl 4-(2,5-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3m**).* Mp. 184 °C. IR (cm^{-1}) 3293, 1701, 1611, 755. ^1H NMR δ 2.35 (s, 3H, 2-CH_3), 2.45–2.85 (m, 5H, *H*-6,7,8 HHQ), 3.65 (s, 3H, COOCH_3), 5.40 (s, 1H, *H*-4 HHQ), 7.00–7.45 (m, 8H, *Ar*), 8.65 (broad, 1H, N*H*). ^{13}C NMR δ 19.0, 34.6, 38.8, 39.2, 43.3, 51.0, 105.6, 111.6, 126.7, 127.2, 127.4, 127.6, 128.9, 130.2, 130.6, 131.2, 132.5, 142.1, 144.0, 146.0, 167.4, 194.9. MS (*m/z*) 296 (%100). Anal. calcd. ($C_{24}H_{21}Cl_2NO_3$) C, H, N.

*4.1.2.6. Ethyl 4-(2,5-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3n**).* Mp. 129 °C. IR (cm^{-1}) 3302, 1696, 1616, 763. ^{1}H NMR δ 1.15 (t, 3H, CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 2.45–2.95 (m, 5H, *H*-6,7,8 HHQ), 4.10 (q, 2H, CH_2CH_3), 5.45 (s, 1H, *H*-4 HHQ), 6.90–7.70 (m, 8H, *Ar*), 8.70 (broad, 1H, N*H*). ^{13}C NMR δ 14.2, 19.2, 34.7, 38.7, 39.2, 43.9, 60.0, 105.6, 111.4, 126.7, 127.2, 128.7, 129.4, 130.4, 131.7, 132.2, 133.0, 142.2, 143.8, 144.0, 145.7, 167.0, 194.9. MS (*m/z*) 310 (%100). Anal. calcd. ($C_{25}H_{23}Cl_2NO_3$) C, H, N.

*4.1.2.7. Methyl 4-(2,6-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3o**).* Mp. 128 °C. IR (cm^{-1}) 3315, 1703, 1615, 764. ^{1}H NMR 2.25 (s, 3H, 2-CH_3), 2.40–2.95 (m, 5H, *H*-6,7,8 HHQ), 3.55 (s, 3H, $COOCH_3$), 5.85 (s, 1H, *H*-4 HHQ), 6.85–7.60 (m, 8H, *Ar*), 8.65 (broad, 1H, N*H*). ^{13}C NMR δ 18.9, 34.7, 38.6, 39.3, 44.0, 50.7, 105.6, 111.6, 126.7, 127.2, 127.4, 127.6, 128.9, 130.6, 131.2, 132.5, 142.1, 144.3, 146.0, 167.3, 194.2. MS (*m/z*) 296 (%100). Anal. calcd. ($C_{24}H_{21}Cl_2NO_3$) C, H, N.

*4.1.2.8. Ethyl 4-(2,6-dichlorophenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3p**).* Mp. 113 °C. IR (cm^{-1}) 3319, 1699, 1615, 769. ^{1}H NMR δ 1.15 (t, 3H, CH_2CH_3), 2.30 (s, 3H, 2-CH_3), 2.40–3.00 (m, 5H, *H*-6,7,8 HHQ), 4.05 (q, 2H, CH_2CH_3), 5.85 (s, 1H, *H*-4 HHQ), 6.90–7.75 (m, 8H, *Ar*), 8.70 (broad, 1H, N*H*). ^{13}C NMR δ 14.1, 19.1, 34.6, 38.9, 39.3, 43.9, 59.7, 102.9, 109.5, 126.7, 127.2, 128.7, 129.4, 130.4, 132.2, 133.0, 142.2, 143.8, 144.9, 152.2, 167.3, 194.9. MS (*m/z*) 310 (%100). Anal. calcd. ($C_{25}H_{23}Cl_2NO_3$) C, H, N.

4.2. Pharmacology

Cadmium chloride, TTX, atropine sulphate, glibenclamide, L-NAME, indomethacin, propranolol hydrochloride, neostigmine hydrochloride, noradrenaline hydrate, pinacidil and DMSO were supplied by Sigma. Stock solution of TTX, cadmium, atropine, L-NAME, propranolol, neostigmine and noradrenaline were dissolved in distilled water. Compounds, pinacidil and indomethacin were dissolved in DMSO. DMSO has no effect on experiments.

New Zealand white rabbits, weighing 2.5–3 kg were used in this study. The study was approved by the Ethics Committee at Gazi University Medical School. Procedures involving animals and their care were conducted in conformity with international laws and policies. At time of study, rabbits were sacrificed with i.v. injection of sodium pentobarbital (30–40 mg/kg, i.v.), followed by removal of the stomach through abdominal incision. The fundal part of the stomach was then dissected parallel to the longitudinal muscle wall. One muscle strip with approximately 15–20 mm length and 2 mm width was obtained and allowed to equilibrate for a period of 60 min in 20 mL organ baths filled with normal Krebs'–Henseleit solution (KHS). The composition of the KHS used in the study was as follows (in mmol/l): NaCl 118; KCl 4.7; $CaCl_2$ 1.26; $NaHCO_3$ 25; Mg Cl_2 0.54; NaH_2PO_4 0.9; glucose 10.04. The solution was gassed with 95% O_2 and 5% CO_2 during the study and temperature was maintained at 37 °C by a thermoregulated water circuit. The pH of the saturated solution was 7.4. Each strip was connected to a force transducer (FDT 10-A, May IOBS 99, COMMAT Iletisim Co., Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA) using software (BSL PRO v 3.6.7, BIOPAC Systems Inc.) which also had the capacity to analyze the data. After mounting, each strip was allowed to equilibrate with a basal tension of 1 g. for 60 min. KHS was replaced with fresh solution every 15 min. during this period. *N*-ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M), indomethacin (COX inhibitor, 10^{-5} M) and propranolol (β-adrenergic receptor blocker, 10^{-6}) were added into the organ bath 20 min before the precontraction in order to eliminate the effects of nitric oxide, prostaglandins and adrenergic agonists. Rabbit gastric fundus smooth muscle strips were precontracted with submaximal concentration of noradrenaline (10^{-5} M). Concentration–relaxation responses for compounds **3a–p**, pinacidil and dimethylsulphoxide (DMSO) were obtained by adding these into the bath in a cumulative manner. A cumulative concentration–response curve was constructed in a stepwise manner after the response to the previous concentration had reached a plateau. This procedure was repeated in the presence of glibenclamide (10^{-6} M).

The relaxant effects of the compounds and pinacidil were expressed as percentage of the precontraction using submaximal concentration of noradrenaline.

In another set of experiments, the inhibitory effects of the compounds and pinacidil were tested on electrical field stimulation (EFS) induced neurotransmitter release. Electrical stimulation of gastric fundus smooth muscle strips was provided by two parallel platinum electrodes (diameter 3 mm, separation 10 mm.). Pulses of 1 ms duration with a voltage of 60 V were delivered by a stimulator (may STPT 03 Research stimulator, COMMAT Iletisim Co., Ankara, Turkey). Frequency–response contractions of gastric fundus smooth muscle strips at 8 Hz. were obtained. To determinate the EFS induced contractile responses TTX (a blocker of Na^+ channels, 10^{-6} M), Cadmium (Cd^{2+}, a voltage-gated calcium channel blocker, 10^{-4} M), atropine (a muscarinic receptor blocker, 10^{-6} M) and neostigmine (acetylcholinesterase inhibitor, 10^{-6} M) were applied (Fig. 1). The EFS induced contractile responses were repeated in the presence of compounds **3a–p**, pinacidil and DMSO at cumulative concentrations (10^{-9}–3×10^{-4} M). The initial response to EFS was used as the control. This experimental protocol was repeated in the presence of glibenclamide (10^{-6} M) (Fig. 2).

4.2.1. Data analysis

The relaxant effects of the compounds on the tissues, precontracted with noradrenaline, were expressed as percentage

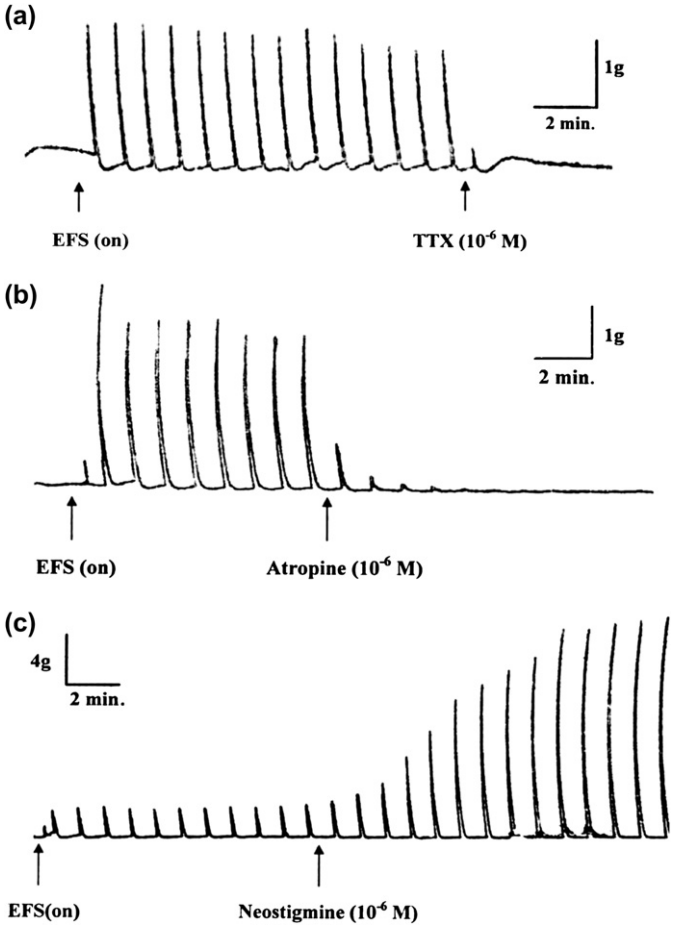


Fig. 1.

of the precontraction using noradrenaline. The inhibitor effects of compounds and pinacidil on EFS evoked contractions (cholinergic neurotransmission) were expressed as percentage of the contraction which was evoked before the application of compounds or pinacidil. To evaluate the effects of the compounds, the maximum response (E_m) [each drug's E_m value has been established at 3×10^{-4} M concentration] and pD_2 values [the negative logarithm of the concentration for the half-maximal response (EC_{50})] were calculated, as predicted from the Scatchard equation for drug–receptor interaction. Agonist pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. While E_{max} is the parameter for efficacy, pD_2 is the parameter for potency. All data are expressed as mean ± standard error.

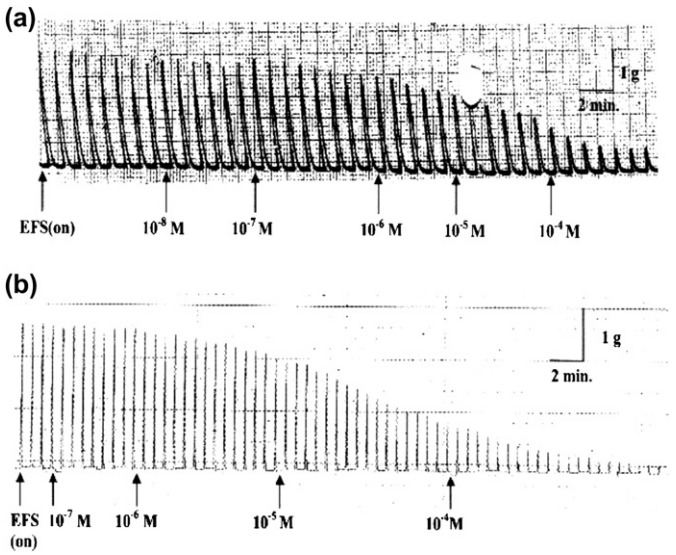


Fig. 2.

4.2.2. Statistical analysis

Statistical comparison between groups were performed using general linear models by Scheffe's F-test and p values less than 0.05 were considered to be statistically significant.

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