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Short communication

Vasorelaxing properties of some phenylacridine type potassium channel openers in isolated rabbit thoracic arteries

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Abstract

In this study, 12 new 2,2,7,7-tetramethyl-9-aryl-2,3,4,5,6,7,9,10-octahydro-1,8-acridindione derivatives were synthesised and their effects on vascular potassium channels and mechanism of induced relaxations on phenylephrine-induced contractile responses in isolated rabbit thoracic arteries was investigated. Pinacidil was used as standard potassium channel openers in this study. Compounds **1**–**12** and pinacidil exerted concentration-dependent relaxation responses precontracted phenylephrine in the aortic rings with the efficacy order: **11** > pinacidil > **7** > **2** > **8** > **3** > **1** > **4** > **10** > **6** > **9** > **5** > **12**. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Potassium channel openers; Acridindione; Synthesis

1. Introduction

Potassium channel opening is a physiological mechanism which excitable cells exploit to maintain or restore at their resting state. Potassium channel openers, which open vascular potassium channels have the potential to restrain or prevent contractile responses to excitatory stimuli or clamp the vessel in a relaxed condition. Glibenclamide, a blocker of ATP sensitive potassium (KATP) channels antagonises these effects. The main vasorelaxant mechanism of the potassium channel openers is to increase the potassium efflux through opening plasmalemmal potassium channels, which repolarises and/or hyperpolarises the membrane. This effect decreases significantly the opening of voltage dependent calcium channels. Reduced calcium release from intracellular sources by the strain agonists through inhibition of inositol triphosphate formation, decreases the sensitivity of intracellular contractile elements to calcium, and accelerates the clearance of intracellular calcium via the Na^+–Ca^{2+} exchanger. Experimental evidence indicates that vasorelaxations induced by potassium channel opening but also another mechanism such as inhibition of the refilling of intracellular calcium stores. Many mammalian aort cells have two distinct ATP-sensitive potassium [K(ATP)] channels. The classic one is in the surface membrane [sK(ATP)] and other is in the mitochondrial inner membrane [mitoK(ATP)]. Cardiac [mitoK(ATP)] channels play a vital role in ischaemic preconditioning and thus represent interesting drug targets [1–4]. They are also important in the control of vascular tone and blood pressure.

1,4-Dihydropyridine (DHP) derivatives and their bicyclo (quinoline) and tricyclo (acridine) analogs are well known group of calcium channel blockers. They are used in the clinic as vasodilator and antihypertensive. 1,4-Dihydropyridine derivatives have also potassium channel opener activities [5–7]. The purpose of this study was to synthesise 2,2,7,7-tetramethyl-9-aryl-2,3,4,5,6,7,9,10-octahydro-1,8-acridindiones and investigate their effects on vascular potassium channels and mechanism of induced relaxations on phenylephrine-induced contractile responses in isolated rabbit thoracic arteries.

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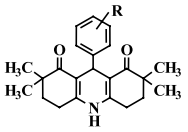
Table 1
Empirical formula, molecular weight, yield (%), melting point of the compounds



Compound	R	Empirical formula	M.W.	Yield (%)	M.p. (°C)
1	H	$C_{23}H_{27}NO_2$	349	76	79
2	2-Br	$C_{23}H_{26}BrNO_2$	428	70	174
3	2-Cl	$C_{23}H_{26}ClNO_2$	383	79	159
4	2-F	$C_{23}H_{26}FNO_2$	367	74	111
5	2-NO_2	$C_{23}H_{26}N_2O_4$	394	51	88
6	2-CF_3	$C_{24}H_{26}F_3NO_2$	415	64	188
7	3-Br	$C_{23}H_{26}BrNO_2$	428	82	136
8	3-Cl	$C_{23}H_{26}ClNO_2$	383	83	155
9	3-F	$C_{23}H_{26}FNO_2$	367	69	120
10	3-NO_2	$C_{23}H_{26}N_2O_4$	394	56	243
11	3-CF_3	$C_{24}H_{26}F_3NO_2$	415	77	91
12	2,3-Cl_2	$C_{23}H_{25}Cl_2NO_2$	418	59	114

2. Results and discussion

2.1. Chemistry

Some properties of the compounds were given in Table 1. Acridine derivatives have been prepared by the reaction of 4,4-dimethyl-1,3-cyclohexanedione with aromatic aldehydes in the presence of ammonia in methanol [8–11] (Fig. 1).

The purity of the compounds was confirmed by TLC. The structure of the compounds was elucidated by IR, ^{1}H-NMR, ^{13}C-NMR and elemental analyses. All spectral data are in accordance with assigned structures. Spectral values of compounds **2**–**12** are not given, because these values are more or less the same as those of compound **1**. In IR spectra, N–H and C=O stretching bands were observed at spectra expected values. In the ^{1}H-NMR spectra, methyl protons were seen at 0.90–1.00 ppm as separated singlet. Aromatic, methylene, methine and NH protons were seen at expected values. The ^{13}C-NMR spectra of the compounds displayed the number of resonance which exactly fitted the number of carbon. Mass spectra of the compounds were taken using the EI technique. Molecular ion peaks were not seen in spectrum. M + 1 ion peaks were observed in spectra of the compounds due to the aromatisation of dihydropyridine ring to pyridine. The base peaks were found by cleavage of the aryl ring from the parent molecule.

2.2. Pharmacology

The relaxant effect of the test compounds **1**–**12** and pinacidil on isolated rings of rabbit aortic smooth muscle were given in Tables 2 and 3. The results of this study indicate that these compounds produced concentration-dependent relaxation in rabbit aortic rings. Only compound **5** displayed very slight relaxant response. Compounds **4** and **6** have the weak concentration-dependent relaxation effect. To investigate whether relaxation induced by the test compounds was due to an interaction with the cyclooxygenase or nitric oxide pathways, tissues were pretreated with indomethacine or L-NAME, respectively. Treatment of rabbit aortic rings strips with these inhibitors did not significantly alter the relaxant activity of compounds **1**–**12**.

In our study, both TEA and glibenclamide affected the relaxant effects or the pD_2 or E_{max} values of the compounds **3**, **9**, **10** suggesting that mediated by K(ATP)- and K(Ca^{2+})-channels. Only glibenclamide decreases the relaxant effects of the pD_2, E_{max} values of the compounds **2**, **4**, **7**, **11** suggesting that it is mediated by K(ATP) channels. However, alternative pathways of relaxation induced by compounds should be investigated such as activation of adenylate or guanylate cyclase, inhibition of cyclic nucleotides, inhibition or activation phosphodiesterases, modulation of Ca^{2+} mobilisation and myosin light-chain phosphorylation.

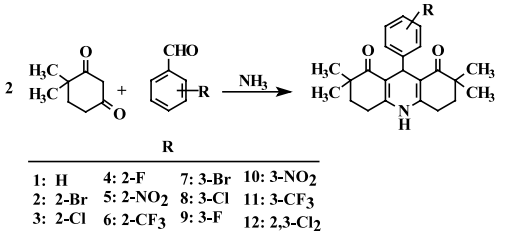


Fig. 1. Synthesis of acridindione derivatives.

Table 2
The relaxant effect of the test compounds **1**–**12** pinacidil on isolated rings of rabbit aortic smooth muscle

Compound	Control	In the presence of glibenclamide	In the presence of TEA
1	87.3 ± 3.7	43.5 ± 6.1*	88.4 ± 4.1
2	98.2 ± 1.7	22.6 ± 4.3*	97.6 ± 2.3
3	92.6 ± 3.1	60.1 ± 4.6*	25.6 ± 4.6*
4	72.1 ± 5.2	17.1 ± 2.6*	68.9 ± 4.6
5	22.6 ± 4.3	23.1 ± 3.2	19.6 ± 4.7
6	58.8 ± 3.8	41.3 ± 4.1*	60.1 ± 4.3
7	99.0 ± 1.0	47.3 ± 6.1*	98.5 ± 1.1
8	98.2 ± 1.1	52.1 ± 4.2*	58.7 ± 5.1*
9	54.2 ± 3.7	53.8 ± 2.4	55.1 ± 4.2
10	64.1 ± 4.5	62.2 ± 3.2	66.3 ± 2.8
11	99.4 ± 0.6	68.7 ± 5.1*	98.6 ± 1.2
12	No effect	no effect	no effect
Pinacidil	99.0 ± 1.0	48.2 ± 3.2*	–

Relaxation is expressed as a percentage of the precontraction induced by phenylephrine (10 μM). The maximal effect (E_{max}, %) value represent mean value S.E. for eight muscle rings for four different preparations. *, $P<0.05$ when compared with control responses (paired t-test).

Table 3
The relaxant effect of the test compounds **1**–**12** pinacidil on isolated rings of rabbit aortic smooth muscle

Compounds	pD_2 ($-\log EC_{50}$)		
	Control	In the presence of glibenclamide	In the presence of TEA
1	3.39 ± 0.04	3.26 ± 0.03	3.41 ± 0.03
2	4.39 ± 0.05	3.92 ± 0.04*	4.41 ± 0.04
3	3.47 ± 0.01	3.20 ± 0.02*	3.06 ± 0.01*
4	3.69 ± 0.03	3.29 ± 0.02*	3.66 ± 0.04
5	4.52 ± 0.06	4.58 ± 0.03	4.54 ± 0.04
6	4.15 ± 0.05	4.21 ± 0.06	4.16 ± 0.04
7	3.79 ± 0.08	3.33 ± 0.04*	3.81 ± 0.07
8	4.09 ± 0.04	4.11 ± 0.05	4.08 ± 0.03
9	3.41 ± 0.01	3.18 ± 0.02*	3.09 ± 0.01*
10	3.39 ± 0.01	3.21 ± 0.02*	3.07 ± 0.01*
11	4.46 ± 0.06	3.86 ± 0.05*	4.48 ± 0.02
12	no effect	no effect	no effect
Pinacidil	4.53 ± 0.07	3.96 ± 0.06*	–

Relaxation is expressed as a percentage of the precontraction induced by phenylephrine (10 μM). The negative logarithm of the concentration for the half-maximal response (pD_2) value represent mean value ± S.E. for eight muscle rings for four different preparations. *, $P<0.05$ when compared with control responses (paired t-test).

Compounds **1**–**12** and pinacidil exerted concentration-dependent relaxation responses precontracted between phenylephrine in the aortic rings with the efficacy order: **11** > pinacidil > **7** > **2** > **8** > **3** > **1** > **4** > **10** > **6** > **9** > **5** > **12**. In control group, it is interesting that compound **11** was more active than pinacidil. Also compound **7** has been found as active as pinacidil. In the presence of glibenclamide, E_{max} values of compounds **3**, **8**–**11** have been found higher than that of pinacidil. It can be said that *meta* substituted derivatives have been found more active than that of *ortho* substituted analogs. When the results of activity studies are investigated in respect to *meta* substituent type, potency order has been found as $CF_3 > Br > Cl > H > F > NO_2$. In addition, it is generally said that the compounds containing 3-substitituted phenyl ring were more active than their 2-substituted analogs. Introduction of a second chlorine atom on benzene ring abolishes the mentioned activity. These findings are in accordance with the structure activity relationships of calcium channel blocking compounds in the series of having 1,4-DHP structure [12].

The endothelium is known to modulate aortic smooth muscle responsiveness to a variety of contractile and relaxant stimuli. To investigate whether relaxation induced by the test compounds was due to an interaction with the cyclooxygenase or nitric oxide pathways tissue were pretreated with indomethacin (10 μM) or L-NAME (30 μM), respectively. Treatment of aortic tissue rings with these inhibitors did not significantly alter the relaxant activity of compounds. This evidence shows that cyclooxygenase and nitric oxide pathways have no roles on relaxant effects of compounds.

To investigate whether relaxation induced by test compounds involved the opening of ATP-sensitive potassium channels and calcium-activated potassium channels, the effects of glibenclamide and TEA were investigated. TEA decreased the relaxant effects and the pD_2, E_{max} values of the compounds **3** and **8** (Tables 2 and 3). Glibenclamide reduced the relaxant effects or the pD_2 or E_{max} values of all compounds except compound **5**. Our results showed that these compounds (except **5**) had a potency for relaxing isolated rabbit aortic smooth muscle, due to opening of ATP-sensitive potassium channels, similar to that of pinacidil.

3. Experimental

3.1. Chemistry

3.1.1. Material and method

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

Melting point: Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA); the values are uncorrected. UV spectra: Shimadzu UV-160A UV–vis Spectro-photometer. IR spectra: Perkin–Elmer FTIR Spectrometer 1720 X (Beaconsfield, UK) (KBr disc) (γ, cm^{-1}). ^{1}H-NMR spectra: Bruker GMBH DPX-400 MHz Digital FTNMR and H^1 AMX 600 MHz FTNMR spectrophotometer (Karlsruhe, Germany) (DMSO-d_6; tetramethylsilane as internal standard). ^{13}C-NMR Spectra: ^{13}C AMX 150 MHz FTNMR 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). Elemental analysis: Leco 932 CHNS-O Elemental Analyser (Philadelphia, USA) (TÜBİTAK, Ankara, Turkey).

*3.1.1.1. 2,2,7,7-Tetramethyl-9-phenyl-2,3,4,5,6,7,9,10-octahydro-1,8-dioxoacridine (compound **1**).* A mixture of 4,4-dimethyl-1,3-cyclohexanedione (0.02 mol), benzaldehyde (0.01 mol) and 10 mL ammonia was heated in methanol for 7 h. Then the solution was concentrated and residue poured in ice-water. The obtained precipitate was crystallised from ethanol. M.p.: 79 °C, yield: 76%.

IR (cm^{-1}): 3210 (N–H), 1707 (C=O), 770, 695 (monosubstituted phenyl). ^{1}H-NMR (ppm): 0.90 (s; 6H; 2- and 7-CH_3), 1.00 (s; 6H; 2- and 7-CH_3), 1.50–2.70 (m; 8H; H$^{3-6}$), 5.90 (s; 1H; H^9), 7.00–7.60 (m, 5H; aromatic protons), 9.0 (s; 1H; NH). ^{13}C-NMR (ppm): 25.0, 25.3, 25.7, 26.0 (acridin 2,2,7,7-CH_3), 36.0, 37.0 (acridin C^{4,5}), 47.0, 48.0 (acridin C^{3,6}), 53.0 (acridin C^9), 59.0, 60.0 (acridin C^{2,7}), 116.0, 117.0 (acridin C^{8a,9a}), 125.0 (phenyl-C^4), 128.0, 128.3, 128.7, 129.0 (phenyl-C^{2,3,5,6}) 139.0 (phenyl-C^1), 146.0, 146.5 (acridin C^{4a,10a}), 203.0, 205.0, (acridin 2 and 8-C=O). Mass (m/z): 350, 335, 317, 304, 291, 281, 273 (%100), 263, 248, 207, 91, 55. Anal. for $C_{23}H_{27}NO_2$ (M.W.: 349) Calc. C, 79.08; H, 7.74; N, 4. 01; Found: C, 78.88; H, 7.43; N, 4.14%.

3.2. Pharmacology

Phenylephrine hydrochloride, *N*-nitro L-erginine methyl ester (L-NAME), indomethacin, tetraethylammonium chloride (TEA), glibenclamide and pinacidil were supplied by Sigma. All drugs were dissolved in distilled water except glibenclamide and compounds (in dimethylsulphoxide = DMSO) an indomethacin (in 1% sodium carbonate). DMSO in organ baths did not affect smooth muscle relaxations induced by compounds. L-NAME was initially dissolved in distilled water and the solution was stored frozen on the day of use it was dissolved and diluted in distilled water. All other drugs were prepared daily.

Mature male albino rabbits weighing 2.5–3 kg were used. At time of study, rabbits were sacrificed with subcutaneous injection of ketamine and xylazine followed by exsanguinations aortic rings of ca. 4 mm. In length were prepared and mounted in 10 ml. Organ baths containing Krebs Henseleit solution of the following composition (in mM): NaCl: 119, KCl: 4.7, $CaCl_2$: 2.5, $NaHCO_3$: 25, MgCl: 1, KH_2PO_4: 1.2 and glucose: 11. The bath solution was maintained at 37 °C and constantly with 5% CO_2, 95% O_2. The pH of the saturated solution was 7.4. After mounting the preparations were allowed to equilibrate for 2 h. During this time the resting tension adjusted to 2 g, a value which was previously found to be optimal for the measurement of changes in tension and solution was renewed every 15 min.

Concentration-relaxation for compounds **1**–**12** and pinacidil were obtained by adding these compounds into the bath in a cumulative manner. These relaxations were compared with those obtained in the presence glibenclamide (ATP-sensitive potassium channel inhibitor) (1 μM) and TEA (a nonspecific calcium-activated potassium channel inhibitor) (500 μM). In another set of experiments, L-NAME (the nitric oxide synthase inhibitor) (30 μM) and indomethacin (PGOx inhibitor) (10 μM) were added into the organ bath 30 min before the precontraction in order to eliminate the effects of nitric oxide and prostaglandins, all of which could have contributed to the aortic smooth muscle relaxation induced by compounds.

The relaxant effects of the compounds were expressed as percentage of the precontraction using phenylephrine. To evaluate the effects of the compounds, the maximum response (E_m) and pD_2 values [the negative logarithm of the concentration for the half-maximal response (EC_{50})] were calculated, as pre-

dicted 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. All data are expressed as mean ± standard error. Statistical comparison between groups were performed using general linear models by *Scheffe's F*-test and *P* values of less than 0.05 were considered to be statistically significant.

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